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BIOLOGICAL BULLETIN

STUDIES ON THE SECONDARY SEXUAL CHARACTERS OF CRAYFISHES: II. FEMALES OF *CAMBARUS VIRILIS* WITH MALE SECONDARY SEXUAL CHARACTERS.

C. L. TURNER.

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During the spring and summer of 1922 and 1923 the writer discovered nineteen specimens of *Cambarus propinquus* that possessed to some degree secondary sexual characters of both sexes. A close examination revealed that all were functional females, some of them bearing eggs when taken but possessing in addition to the usual female secondary sexual characters from one to three male secondary sexual characters as well. A number of males were found bearing extra male characters and two females with extra female characters. These results led to the examination of a fairly large collection of *Cambarus virilis* which had already been made and also to the search for aberrant conditions whenever specimens came to hand.

Next to *Cambarus propinquus* *Cambarus virilis* is the most abundant crayfish in southern Wisconsin and the two are usually taken together in the lakes and streams.

INTERSEXES IN *Cambarus virilis*.

Specimens from Delavan Lake.

Intersexes in *Cambarus virilis* were first found in Delavan Lake on June 10, 1923. Subsequent collecting trips were made on June 11, June 14, June 30 and November 20. On June 10 fifty-seven mature females were taken, of which thirty-eight were carrying eggs. Thirty-seven of those carrying eggs had in addition to a full complement of female secondary sexual characters

copulatory hooks on the third segments of the third walking legs as fully developed as those of the normal males (Fig. 1, *A*, *B*, *C*).

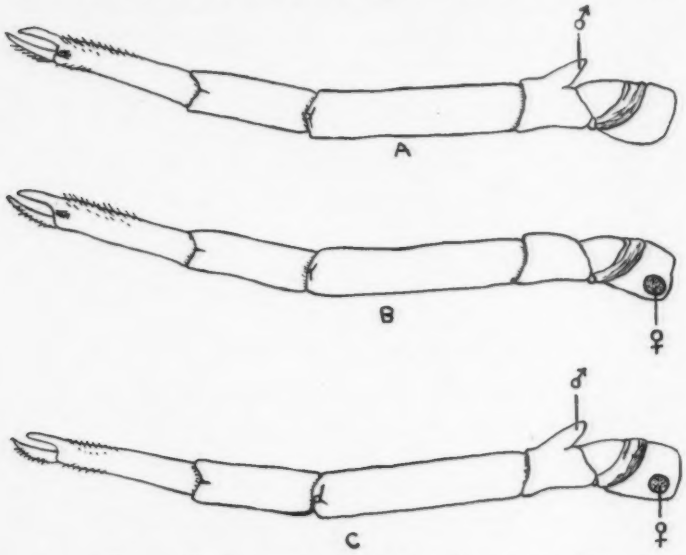

FIG. 1. Diagram illustrating the male copulatory hook on the third walking legs of a female.

A. Normal male walking leg with male copulatory hook. ♂.

B. Normal female walking leg with oviducal opening. ♀.

C. Intersex condition with both copulatory hooks and oviducal openings. ♂♀.

One of the specimens carrying eggs was a normal female. Of the remaining eighteen which did not carry eggs fourteen had well-developed hooks on one or both third walking legs. A number of specimens under 34 mm. in length were taken but they were too juvenile to have the hooks developed.

On June 11 six additional females were taken, three of which carried eggs. All possessed the copulatory hooks of the normal male and one large specimen had in addition a second set of secondary sexual characters in the form of small but distincly male-like appendages which replaced the usual rudimentary female appendages of the first abdominal segment (Fig. 2, *A*, *B*, *C*). The appendages resembled rather closely the normal appendages in their proximal halves but the usual slender tips of

the normal male appendages were lacking and the appendages were only about half normal size.

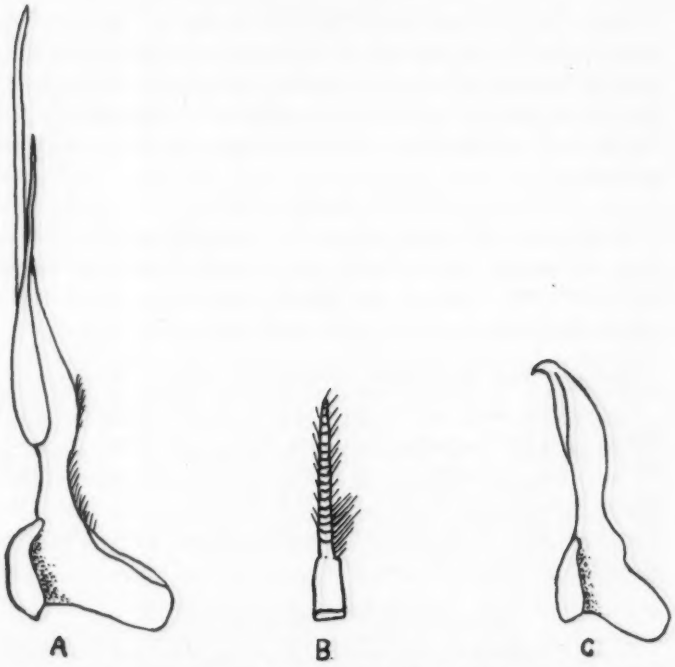

FIG. 2. Diagram illustrating the character of the first abdominal appendage in one of the intersexes.

A. Normal male appendage of the first abdominal segment.

B. Normal rudimentary female appendage of first abdominal segment.

C. Small but male-like appendage occurring on the first abdominal segment of one of the intersexes.

On the subsequent dates specimens were collected from various parts of the lake with results agreeing essentially with those first obtained. In all, over one hundred aberrant females were preserved and many more were examined and recorded. Upon the basis of the data gathered it was estimated that 88 per cent. of all the females of *Cambarus virilis* in the lake possessed at least one set of male secondary sexual characters.

Over two hundred males of both form I. and form II. have been

examined and all have displayed only normal secondary sexual characters.

Specimens from Turtle Creek.

Since Turtle Creek forms the only outlet of Delavan Lake special attention was given to this creek and collections were made from the creek at three localities about eight miles apart. Of 263 females of *Cambarus virilis* collected a single one had the copulatory hook on the left third walking leg and was otherwise normal.

Specimens from Ponds.

A collection which was made in the spring and summer of 1922 from the ponds about Beloit was examined and out of 309 specimens (137 females) one female was found which had a copulatory hook on the left third walking leg.

Specimens from Rock River tributaries exclusive of Turtle Creek.

Collections were made from four small tributaries of Rock River, all within a radius of twenty miles and all females taken were normal. A collection of eighty females taken from Sugar River, a larger tributary of the Rock River, contained two aberrant female specimens each of which bore a copulatory hook on one of the third walking legs.

Specimens from Northern Wisconsin.

A small collection purchased from a supply by the University of Wisconsin contained sixty females two of which had copulatory hooks on both third walking legs. Through correspondence with the supply company it was learned that the specimens came from somewhere in northeastern Wisconsin.

SUMMARY OF FEMALES WITH MALE CHARACTERISTICS.

1. In Lake Delavan 88 per cent. of the females of *Cambarus virilis* displayed at least one set of male characters, namely, male copulatory hooks on the third walking legs.

2. An old female from Lake Delavan carried in addition to the copulatory hooks a pair of small but distinctly male-like appendages upon the first abdominal segment.

3. Outside of Lake Delavan six females have been collected which had copulatory hooks on one or both third walking legs.

These aberrant females totalled about 1.5 per cent. of all the females collected outside of Lake Delavan.

Discussion.

From the data cited above it is evident that the presence of male secondary sexual characters in females of *Cambarus virilis* is not uncommon in the waters of Wisconsin. The exploration so far has been very limited but from the evidence already gained it seems quite probable that other localities may be found which will contain as large a proportion of aberrant females as have been found in Lake Delavan. It may be that sex intergrades in *Cambarus virilis* may be much more common generally than is supposed to be the case.

The small number of intersexes in *Cambarus propinquus* and in *Cambarus virilis* outside of Lake Delavan makes it possible to approach the question as to the possible causes for the intersexes in an indirect and hypothetical way but the very high proportion of intersexes in *Cambarus virilis* found in Lake Delavan should afford a much more satisfactory basis for such a study.

After observations and experiments upon intersexes in Fiddler Crabs Professor Morgan [1] reviews the possible causes for intersexes in Arthropods in general and more particularly in the Crustacea. He points out six possibilities. (1) The intersexes may be true hermaphrodites, possessing to some degree both ovaries and spermaries. (2) Parasitism affecting the host directly may be responsible for the intersexes. (3) Parasitism of the gonads may cause a loss of the supposed control of the direction of development of the secondary sexual characters. (4) The intersex condition may be due to some environmental influence. (5) Changes in genetic constitution may account for the condition. (6) Peculiar or accidental embryonic development may be the cause.

Of the above possibilities the third and the fifth have been considered in connection with intersexes in *Cambarus propinquus* in another paper.[2] It was borne in mind that the evidence

[1] Morgan, T. H., 1920, *American Naturalist*, Vol. 54, pp. 220–246.

[2] Turner, C. L., 1924, "Studies on the Secondary Sexual Characters of Crayfishes. I. Male Secondary Sexual Characters in Females of *Cambarus propinquus*," Biological Bulletin, Vol. XLVI., No. 6.

obtained thus far in the Arthropods does not favor the assumption of control of the direction of development of the secondary sexual characters by secretions from the gonads. However, the prevalence of parasitism in the forms studied could not be overlooked and parasitism of the gonads was stated as a possible cause for the intersex condition. More weight was attached to the possibility of genetic disturbances being the important factor for reasons to be stated later. In the aberrant specimens described here parasitism was variable in its occurrence among the intersexes even to the point of being non-existent in a considerable number. Parasitism, either as affecting the body directly or the secondary sexual characters indirectly through the gonads cannot, therefore, be considered seriously.

If the specimens described here were truly hermaphroditic it should be possible to find some traces of testicular tissue within or near the ovaries. All specimens seem to have been functional females and at a season when they might be expected to carry eggs or embryos they were doing so. A considerable number of specimens have been carefully dissected and macroscopic and microscopic examinations made but with the result of finding nothing that might be called testicular tissue. Testicular tissue may be present but it has not been found as yet.

The possibility that some environmental influence such as peculiarities of temperature or chemical substances in solution may have been causing the aberrancies can be tested by removing specimens from Lake Delavan while they are still in the egg stage and rearing them under changed conditions. Such a study is on the program for the coming spring.

Peculiar or accidental embryonic development without attendant environmental influences to account for them might be considered to account for a small number of intersexes but this factor could scarcely be invoked to account for as large a proportion as 88 per cent.

The suggestion that changes in genetic constitution have been the underlying cause seems by the process of elimination to be the logical choice and indeed there is considerable direct evidence to support such a claim. This possibility was suggested in the cases of *Cambarus propinquus* previously described for the reason that intersexes from the same localities showed a remarkable degree of

similarity from which it could be inferred that they might have been members of the same brood. The evidence from Lake Delavan for the inheritance of the intersex condition lies in the fact that at least three generations of crayfishes were represented in the specimens secured. If the peculiar condition were being transmitted it should be expected that the same proportion of intersexes would obtain in specimens representing different ages. This was found to be the case. Admitting that constant but peculiar environmental conditions might be a possible cause it is strongly suspected that the cause for the intersex condition lies in some change in the genetic constitution, possibly in the chromosome complex and that the condition is being inherited. It is proposed to test this inference by collecting intersex females which are bearing eggs and to rear the eggs to maturity or to such a time as it will be possible to determine whether or not the young possess the peculiarities of the mother.

STUDIES ON THE SECONDARY SEXUAL CHARACTERS OF CRAYFISHES: III. MALES WITH SUPERNUMERARY MALE SECONDARY SEXUAL CHARACTERS IN THE SUBGENUS *FAXONIUS*.

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In the genus *Cambarus* the males of the subgenus *Cambarus* are distinguished from the males of the other subgenera by the presence of copulatory hooks upon the third segments of the third and fourth walking legs. The other subgenera possess these copulatory hooks only upon the third walking legs. These hooks are secondary sexual organs and are used to some extent in the process of copulation. It has been supposed that the number was quite constant and the few specimens that have varied from the normal condition have been classed as "freaks." Ortmann[1] in discussing the use of the copulatory hooks as diagnostic features says of *Cambarus virilis* and of *Cambarus propinquus* ". . . always only the third pereiopods with hooks, barring freaks."

A large number of individuals, indeed, enough to constitute a considerable proportion of a crayfish population have been found in *Cambarus virilis* and in *Cambarus propinquus* to deviate from this rule in the possession of more than one pair of hooks. These specimens can scarcely be classed as freaks. The deviation becomes the more interesting since it occurs in a crayfish population which has a large proportion of other aberrant conditions in the secondary sexual characters such as the occurrence in females of male secondary sexual characters, the occurrence in males of female secondary sexual characters and the occurrence in females of extra female secondary sexual characters.

The thirty-two specimens to be described have been taken with the exception of three from the lakes and streams of Wisconsin,

[1] Ortmann, A. E., 1905, "The Mutual Affinities of the Species of the Genus *Cambarus* and Their Dispersal Over the United States," *Proc. Amer. Phil. Soc.*, Vol. 44, pp. 91–136.

It seems likely that the survey need only be extended to discover many more specimens since the present ones were taken in a very limited bit of collecting.

Description of Specimens.

1. *Specimens of Cambarus virilis.*

Two specimens have been taken from the tributaries of the Rock River in the vicinity of Beloit, Wisconsin, both bearing in addition to the copulatory hooks of the third walking legs a second pair of such hooks on the second walking legs. The additional hooks were slightly smaller than the normal ones but were distinct. Both specimens were about two years old. They represented a proportion of about .5 per cent. of all the males taken.

A collection of large specimens examined at the University of Wisconsin and later traced through a supply company to north-eastern Wisconsin as the locality from which the collection came contained forty-eight males of which sixteen had copulatory hooks upon the second walking legs in addition to those of the third walking legs. Two other males in this same collection had each a single additional hook upon the left second walking leg. The additional hooks were in some cases as well developed as those normally occurring upon the third walking legs but in most instances they were smaller. In this same lot of specimens there were 3.5 per cent. of females which possessed each a well-defined male characteristic in the form of a pair of copulatory hooks on the third walking legs.

2. *Cambarus propinquus.*

In this species the occurrence of males with additional male characteristics has been found to be rather constant but in no locality has there been so large a proportion of aberrant individuals as in *Cambarus virilis*. There is, however, a greater variation in the additional characters. In some instances the additional hooks are found upon the fourth walking legs and in others upon the second walking legs.

During the summer of 1922 considerable numbers were collected from time to time from Turtle Creek but only two males were noticed which had extra copulatory hooks. In one

instance the additional hooks were carried upon the fourth walking legs and in the other upon the second walking legs.

In July, 1923, a collection was made from a tributary of Turtle Creek twelve miles from the first locality. Of 150 males five had additional copulatory hooks. Three of these had well developed additional hooks upon the second walking legs while the other two specimens bore the extra hooks upon the fourth walking legs.

A single specimen from a collection of twenty-five males from Delavan Lake had extra copulatory hooks upon the fourth walking legs.

A collection of 152 male specimens from a tributary of Rock River twelve miles south of Turtle Creek contained three with extra male hooks, two of them bearing the extra hooks upon the second walking legs and the other bearing them upon the fourth walking legs.

3. *Cambarus obscurus.*

A small collection of thirty-eight males from Ashtabula, Ohio was examined at Ohio State University and one male was found with extra copulatory hooks upon the second walking legs.

4. *Other Species of Cambarus.*

An examination of 1280 specimens from eight additional species of *Cambarus* failed to bring to light any new aberrant specimens.

Discussion and Summary.

The records cited above are quite limited but a few general facts become apparent when they are examined critically.

1. Supernumerary male secondary sexual characters seem to differ with the species in which they occur. An examination of 600 specimens of *Cambarus clarkii* failed to reveal any deviations from the usual male secondary sexual characters either in the copulatory hooks upon the third and fourth walking legs or in the modified appendages of the first and second abdominal segments. An examination of 310 specimens from three species of the subgenus *Faxonius* likewise failed to show any aberrant features. On the other hand there is a rather constant occurrence of additional copulatory hooks in *Cambarus propinquus* as represented by specimens from southern Wisconsin, northern Illinois

and northern Ohio. About 2 per cent. of all the males of *Cambarus propinquus* examined have been aberrant in this respect. In *Cambarus virilis* the general occurrence of specimens with supernumerary sexual characters is less but locally the proportion of specimens abnormal in this respect mounts up to 33 per cent.

If the cause or causes underlying the peculiar condition is to be studied to advantage it would be in localities in which the proportion of peculiarites was remarkably high.

2. The manner in which specimens with supernumerary hooks vary from the normal seems to have a certain degree of constancy in a given species. In *Cambarus virilis*, for example, the deviation is always in the direction of the development of additional hooks upon the second walking legs. In *Cambarus propinquus*, on the other hand, the additional hooks may be developed upon either the fourth or the second walking legs but never upon both the fourth and the second walking legs in the same specimen.

3. Deviation has thus far always been in the direction of more than the normal number of copulatory hooks and not less than normal. This has held for all species examined whether they normally possess copulatory hooks upon the third and fourth walking legs, as in the case of *Cambarus blandingii* acutus and *Cambarus clarkii*, or upon the third walking legs only as in all species of the subgenus *Faxonius* and the subgenus *Bartonius*.

4. It is suggested that, in *Cambarus propinquus*, since the second, third and fourth walking legs are potential bearers of copulatory hooks a specimen may be found expressing the extreme degree of deviation from the normal condition and bearing copulatory hooks upon all three of these walking legs.

FORMATION OF FOOD-CUPS IN *AMŒBA* INDUCED BY CHEMICALS.

J. GRAHAM EDWARDS.

From the Zoölogical Laboratory, The Johns Hopkins University.

Feeding reactions of *Amœba* have been described by different investigators and from various standpoints. Jennings (1), Kepner and Taliaferro (3), Mast and Root (4) Schæffer (5), and Beers (6) have contributed valuable data concerning such reactions. Jennings mentions in connection with the extension of a pseudopod toward a food particle, that this should be attributed partly to chemical stimulation. Mast and Root hold that surface tension is probably an insignificant factor in the process of feeding, and Schæffer is of the opinion in this connection that the presence alone of a substance in solution is not sufficient to attract *Amœba*, or to cause ingestion, but that the substance must be actively diffusing from a definitely localized region. Kepner and Edwards (2) conclude that there is no hypothesis as yet advanced to explain quantitatively the reaction involved in the movement of Rhizopoda, which can be applied to *Pelomyxa's* movement around food-bodies.

While locomotion and movements observed in feeding still remain unexplained, the following observations of feeding reactions induced by chemicals may further extend or limit this problem. For feeding reactions quite comparable in every way to those described by the investigators mentioned, occur without the presence of food and in a homogeneous medium. There are no visible stimuli.

When amœbæ are washed in three changes of distilled water and immersed in certain chemically pure salt solutions, food-cups are formed in the absence of solid particles which are comparable to those observed in the process of ingesting food. These cups are formed as follows: On the under surface of one or more pseudopods a local expansion of the ectoplasm occurs accompanied by an inflow of endoplasm. Immediately the marginal surface of

the protuberance formed by this local expansion becomes firmly attached to the substratum and the enclosed area arches upward causing much of the endoplasm between the upper and lower surface to recede: thus forming a spoon- or cup-like concavity. The marginal surface of this concavity draws slowly together from all directions reducing the aperture of the concavity until it is entirely closed. A vesicle is thus formed not unlike the

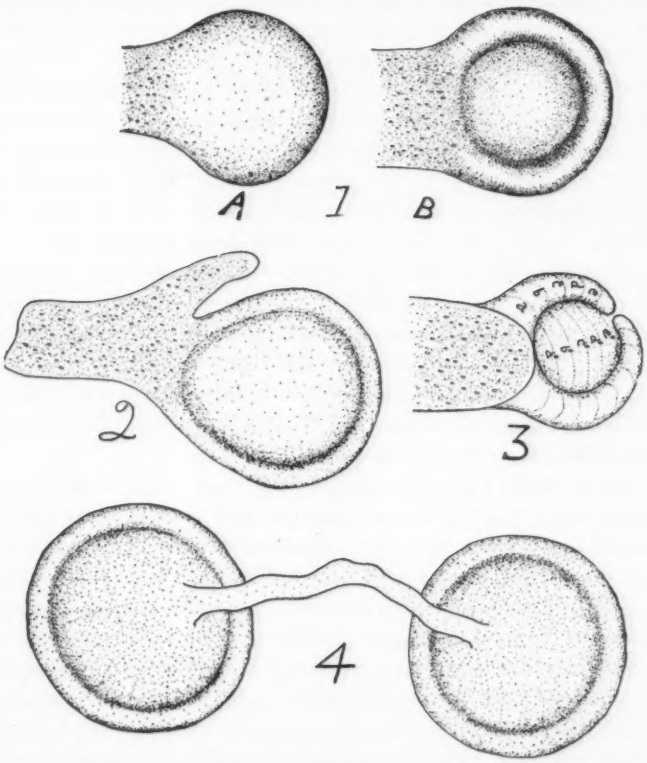

FIG. 1, *A* AND *B*. Free hand sketch of the pseudopod of an *Amœba* during the formation of a food-cup.

FIG. 2. A similar sketch of a food-cup which involves almost the entire *Amœba*.

FIG. 3. Another type of food-cup formation in which two accessory pseudopods arise and advance consecutively through positions indicated by *a*, *b*, *c*, *d*, *e* followed by an overarching sheet of ectoplasm indicated by a^1, b^1, c^1, d^1, e^1.

FIG. 4. In this type of food-cup formation the two cups formed are connected by a strand of protoplasm.

contractile vacuole in appearance (Figs. 1, 2, 3 and 4). This vesicle is then constricted off from the ectoplasm and borne along in the endoplasmic current as a normally formed food-vacuole. The size of the concavity varies depending apparently on the size of the pseudopod giving rise to it. If the amœba is monopodal, the concavity and vesicle are large; if heteropodal, they are smaller. The upper as well as the lower surface may develop such a concavity and vesicle so that contact with the substratum is not necessary. The food-cups thus formed rarely remain visible for longer than 3 to 5 minutes.

The most favorable media for inducing these feeding reactions are as follows: A mixture of NaCl, LiCl and $CaCl_2$; NaCl alone or mixtures of neutral sodium salts; $CaCl_2$ and a mixture of NaCl with $CaCl_2$; or $NaNO_3$ mixed with $Ca(NO_3)_2$. Mixtures of the different salts used were always of the same concentration and in equal parts. Suitable concentrations are $N/300$ or $N/500$. No appreciable difference exists in either concentration as to the size or number of food-cups formed. Sea-water diluted by adding two parts of distilled water to one of sea water is also an excellent medium for such feeding reactions. Less favorable media are KCl, $N/500$, and mixtures of potassium salts, $MgCl_2$ and $MgSO_2$. In neutral ammonium salts and in $SrCl_2$ and $BaCl_2$, $N/500$, these reactions occur only occasionally.

In the most favorable mixture, *i.e.*, NaCl, LiCl and $CaCl_2$, containing a hundred or more amœbæ, this reaction is observable within one or more hours in a considerable number of specimens and thereafter almost continuously in now one group, now another. In a few hours it is possible to obtain as many amœbæ as desired in all of the stages manifest in this reaction. Owing to the rigidity of the marginal surface of the food-cup, amœbas can be removed from the solution with a pipette and immersed in a fixing fluid without appreciable change in the character or form of the food-cup. Examination of food-cups when amœbæ are in the solution as well as after they are fixed and sectioned fails to show the presence of any particle or other visible stimulus normally present in feeding reactions.

Feeding reactions also occur in a dilute acid or base, *e.g.*, HCl or NaOH if a neutral salt, *e.g.*, NaCl is added. Feeding reactions were not observed to occur in other than neutral salt solutions

except to a slight extent in $NaHCO_3$. It is this condition that shows the probable significance of the pH of the medium in conditioning such reaction and the effect of the medium on the surface. In general, solutions impeding or preventing locomotion do not induce feeding reactions.

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THE STRUCTURE OF THE UNDULATING MEMBRANE IN THE CILIATE *BLEPHARISMA*.

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It has for long been generally accepted [1] that the undulating membrane of ciliates is composed of a serried row of fine cilia fused into a homogeneous-appearing membrane. This conception has been based largely on the fact that the membrane, when treated with fixatives, resolves itself into a linear series of very fine fibrillæ. Moreover, several investigators have remarked on the occasional appearance of delicate striations in the living membrane of certain ciliates. In addition to this Maier reports having noticed, in the living specimen an actual breaking down of the membrane into fibrillæ for some distance inward from its free border.

It was thought of interest to study the behavior of the undulating membrane in *Blepharisma undulans* by subjecting it to manipulaton with the microdissection needle.

A single blepharisma, in a shallow hanging drop in a Barber's moist chamber, was held stationary by means of a microneedle

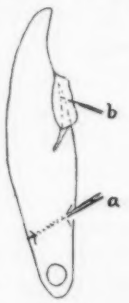


FIG. 1.

thrust into the posterior end of the body, Fig. 1, *a*. Another needle, Fig. 1, *b*, having a very slender and long tapering tip (the

[1] For a review of the literature on this subject see H. N. Maier, "Der feinere Bau der Wimperapparate der Infusorien," *Arch. f. Protistenk.*, 2, 73 (1903).

needle being about one micron in diameter at about five micra back from the tip) was carefully brought up to the free margin of the undulating membrane. By this means the membrane could be considerably deflected without being torn. The undulations continued in spite of the forced position of the membrane.

With the needle it was found possible to press down upon the membrane and to pierce it, Fig. 2, *a*. The membrane at once split vertically along a line running through the puncture, Fig. 2,

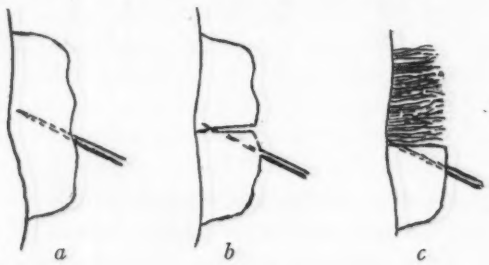


FIG. 2.

b. The part of the membrane beyond the needle then rapidly broke into a linear series of very fine cilia which were plainly visible because they were now beating out of unison Fig. 2, *c*. As long as the needle was kept in position the cilia of the disrupted portion of the membrane repeatedly fused and separated again. The fusion was partial, being limited to several groups of the cilia which repeatedly kept fusing and then breaking apart.

When the needle on the membrane was removed the entire row of cilia quickly fused and reconstituted the membrane. By less delicate treatment with the needle the entire membrane could be broken up momentarily into cilia. Upon removal of the needle the cilia always reunited to form an intact, homogeneous membrane. A membrane could thus be repeatedly disrupted and then allowed to resume its original normal aspect without any sign of having been injured. This is indicated by the fact that when the animal is freed by removing the transfixing needle it will swim away with the membrane undulating normally.

A temporary, partial breakdown of the undulating membrane was also observed to occur spontaneously while under dark-field illumination. The picture was most striking for the membrane,

while intact, could be detected only because of its shimmering and silvery outline. Suddenly, the membrane was seen to break up into innumerable cilia beating out of unison. A few seconds later the brilliantly lighted cilia disappeared as they fused into an optically structureless membrane.

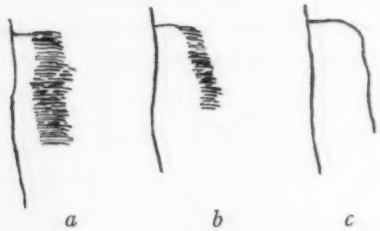


FIG. 3.

It is significant that the reconstitution of the membrane occurs by a fusion which spreads from the bases of the cilia toward their free ends. Three successive stages of this process are shown in Fig. 3, *a*, *b* and *c*. This suggests the possibility of a slime-like substance being secreted and spreading between and over the cilia to join them into an optically homogeneous sheet.

THE EYE AND OPTIC TRACT IN NORMAL AND "EYELESS" *DROSOPHILA*.

MILDRED HOGE RICHARDS AND ESTHER Y. FURROW.

(Contribution from the Zoölogical Laboratory, University of Oklahoma, Second Series, No. 48.)

One of the most interesting mutations that has occurred in *Drosophila melanogaster* is known as "eyeless." It was first observed by the senior author in 1913, when some flies with eyes entirely wanting on one or both sides appeared in the cultures. The inheritance of eyeless was worked out at that time and the gene which is responsible for the condition was located in the fourth chromosome. The stock breeds true to the eyeless condition, but there is no fixed type of eyeless. It seems very probable that while the known gene in the fourth chromosome determines that the eye shall be reduced, other genes not yet located govern the extent of the reduction of the eye.

Recently Little and Jones have carried on a selection of eyeless flies in order to obtain races showing large and small proportion of eyeless. Some progress was made and races of these two kinds were separated. These results may indicate a genetic difference between the two lines.

The name "eyeless" was perhaps incorrectly applied, since all the flies of the pure stock are not entirely without eyes. There is considerable variation in the size of the eye. Zeleny has found that the eye of a normal fly of certain strains which he had under investigation contains from 800 to 840 ommatidia. In the eyeless stock the eye may be totally wanting, or it may appear superficially to be normal in every particular except size, even at its maximum being smaller than the eye of the normal wild type. Between these two extremes all gradations appear. Furthermore there is usually in eyeless an entire lack of symmetry between the eyes of the two sides. Sometimes the eyes are about equal in size, sometimes present on neither side, and sometimes there will be a fair sized eye on one side while on the other there will be only a small one or none at all.

A superficial examination of the heads of eyeless flies revealed the fact that those totally eyeless have no external trace of ommatidia. An interesting question, first suggested by Dr. T. H. Morgan, arose concerning the condition of the optic tract in the totally eyeless flies. The heads of such flies are greatly reduced in size resembling pin heads. Since the eye is gone and the head so greatly reduced, it would seem highly probable that a large part of the optic tract may be wanting. Accordingly a comparative study of the heads of normal and eyeless flies was undertaken. Sections were cut and a special study made of the optic tract. No attempt was made to study the brain nor was the optic tract studied neurogically. This had been done for the bee by Kenyon. The problem was to compare the optic tracts of normal and eyeless strains. In other words what part of the optic tract is missing in eyeless?

TECHNIQUE.

The flies were anæsthetized with ether. Then with the aid of a lens the heads were cut with a razor blade from the body so as to include most of the thorax as well as the head, since it was found that subsequent handling was considerably facilitated by the presence of the thorax. The desired portion thus cut off was immediately immersed in the killing fluid. Several killing fluids were tried but the most satisfactory was Bouin. Any picric acid combination proved satisfactory, and considerable success followed the use of a warm saturated solution of picric acid in 50 per cent. alcohol. Material was allowed to remain in Bouin 24 hours. It was then dehydrated and embedded in paraffin. Sections were cut at various thicknesses, 5, $7\frac{1}{2}$, 10, and 15 microns. For general study the thickness of 10 microns was most practical. Both hæmalum and iron hæmatoxylin were used as stains. For detailed work the latter was more satisfactory and was used extensively throughout the study. In a few cases eosin was used as a counterstain.

DESCRIPTION.

The eye of *Drosphila* is very large and occupies almost the whole side of the head (Plate III., Fig. 1). Our sections revealed the structure of the eye. It is of the large compound type characteristic of insects. The whole eye with its most distal

ganglion, called in this paper the outer (periopticon of Hickson) has in general the shape of a cone of rather low altitude with the apex directed inwards. The surface of the eye is covered with small facets between which project hair like bristles. (Plate I., Fig. 1, and Plate III., Fig. 2.) Each facet is the outer portion of a single ommatidium. In the center of the eye the ommatidia are at right angles to the basement membrane but at the sides of the eye they join the membrane at a considerable angle. Beneath the layer of facets is a layer of pseudocones or lens-like structures. In the preparations there is often an artificial separation between the facets and the pseudocone layer. Immediately under the pseudocones there is a layer of retinulæ and rhabdomeres. The latter are darkly stained rods which stand out clearly in our sections. Under this layer is the basement membrane which separates the eye from the outer ganglion.

A closer study of the eye under a higher magnification reveals more clearly the nature of each ommatidium. Each pseudocone appears as a hollow space on whose proximal side are two of the four cone cells which produce it, with their contained nuclei (Semper's) (Plate I, Fig. 2). The pseudocone is characteristic of flies with short antennæ, and is in contrast with the eucone in which the nuclei are distal to the lens. Beneath each pseudocone is a spindle-shaped group of rods or rhabdomeres. Outside each rhabdomere is an elongated cell or retinula. Outlines between the retinulæ do not show clearly in most of our longitudinal sections, but between retinulæ of adjacent ommatidia, pigment cells can be seen. At the ends of the spindle the rhabdomeres are close together, while at the middle of it they are farther apart. In each ommatidium there are seven retinulæ and seven rhabdomeres. Six of the latter are approximately equal in size, but the seventh is smaller and seems to be joined edgewise to a small lamella. Plate I., Fig. 3 shows a cross section of an ommatidium near the distal end. Here the seven rhabdomeres are close together, and the outlines of the seven retinula cells, some of which show nuclei, can easily be made out. Plate I., Fig. 4, shows a cross section of the ommatidium proximal to that of Fig. 3. Here the rhabdomeres are farther apart and the spindle-shaped ommatidium has widened. The retinulæ are much smaller in section and appear as a protoplasmic circle around the rhabdo-

meres. Indentations in this protoplasmic ring reveal the position of the boundaries of the retinulæ. Each retinula has, as the cross sections indicate, a nucleus at the distal end just below the nucleus of the cone cell. Nuclei were also observed at the proximal end of the retinulæ, although we were not able to determine with accuracy whether each one has a nucleus at this level.

Pigment cells separating the ommatidia were distinguished in both longitudinal and cross sections. These separate the cone cells and the retinulæ of the neighboring ommatidia. Since the pigment cells did not seem to have a practical bearing on our problem, no attempts were made to demonstrate them with special methods by which a more complete study of them would be possible.[1] Hence the drawings do not include them.

The eye is connected to the brain by a series of three ganglia which we will call the outer, median, and inner; these correspond to the same ganglia named by Hickson from the outside inward as periopticon, epiopticon, and opticon respectively. The outer ganglion joins at its distal edge the basement membrane and is flattened out to conform to the shape of the eye. Centrally it narrows passing into nerve fibers which decussate to enter the median ganglion (Plate II., Fig. 1). The median and inner ganglia have each the shape of a meniscus lens in transverse sections. From the median ganglion fibers pass to the inner. The inner ganglion is connected to the brain by short fibers which do not seem to cross. There is thus no optic nerve as in crustaceans, a fact long ago pointed out by Berger.

A more detailed study of the outer ganglion (Plate I., Fig. 5) shows that it consists in its proximal part of nerve fibers arranged in groups in parallel fashion. These groups constitute the elements of the outer ganglion. In the distal part among cells which do not seem to be uniformly arranged are scattered tracheal vessels and groups of fibers which reach to the basement membrane. The connections between these fibers and the elements of the ganglion could not be followed in our sections.

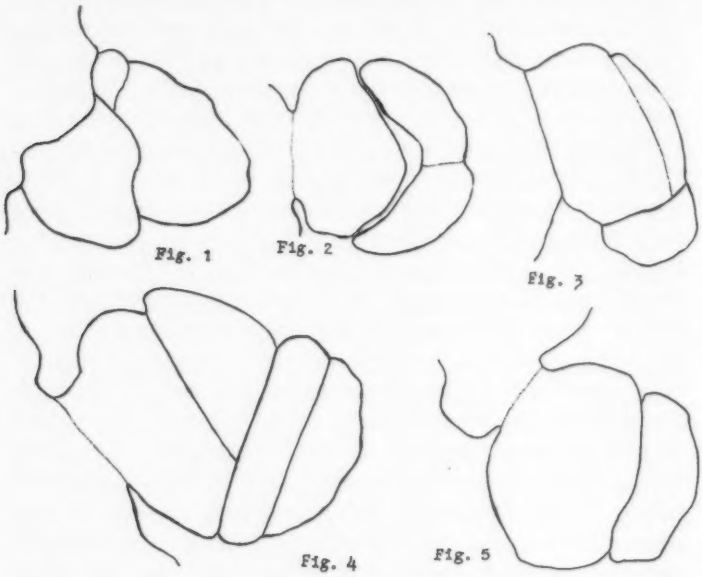
When the heads of eyeless flies were studied certain facts

[1] While this paper was in proof, a study by O. A. Johannsen, entitled "Eye Structure in Normal and Eye-Mutant Drosophilas" appeared: Jour. Morph. Vol. 39, no. 2. Dec. 1924. The pigment cells are treated in detail in this work.

became at once evident. Many of the eyes of the eyeless stock were simply reduced in size. Most of these reduced eyes have a large number of ommatidia (Plate II, Fig. 2), but obviously even the larger eyes have a smaller number than have the normal. Some of the eyes, however, are very small, sections through the middle showing scarcely half a dozen ommatidia. Plate III., Figs. 3 to 8 are microphotographs through heads of eyeless flies. In Fig. 3 there is a small eye on the right side but none on the left. On the right side the three optic ganglia are present, for a higher magnification reveals the fact that the middle ganglionic mass here shown is duplex, representing both the median and the inner ganglia. Fig. 4 shows a head with a small eye on each side. On the left side the outer ganglion is somewhat rounded, and the mass of fibers connecting it to the median ganglion is larger in proportion than that of the normal eye. Below this mass of connecting fibers may be seen the median and inner ganglia, each a double lens-like structure as in the normal eye. The section does not go through the optic tract of the right side. Figs. 5 and 6 show each a fair-sized eye on the right side and also the three ganglia of the right optic tract. In Fig. 5 the section goes through but a small portion of the brain. Fig. 7 shows a smaller eye on the right side and all three optic ganglia, although in the picture the median and inner are so close that they seem to form a single mass. These and other sections show that where the ommatidia are present at all they seem to have the normal structure and all three of the optic ganglia are present though in many cases they are reduced in size corresponding to the size of the eye.

In the totally eyeless flies, that is, in those flies that showed no external trace of ommatidia, the outer ganglion was also absent (Plate II., Fig. 3). This most distal ganglion had disappeared as had the eye itself. The other two ganglia, however, were present, though much contracted and in close apposition. This is well illustrated by the text-figures 1 to 5 which are outlines of the median and inner ganglia in the totally eyeless condition. These two ganglia have become a rounded, more or less shapeless mass, and it is only with high magnification that the division between them can always be made out. A close study of the ganglia, however, always revealed the fact that the mass is

duplex, representing both median and inner. Sometimes one of the ganglia, as in Fig. 3, will show the double lens-like structure



FIGS. 1–5.

best seen in transverse sections. In Fig. 4 each of the two ganglia show this double nature, similar to that of the normal eye. The contraction in the two inner ganglia in part explains the great reduction in the size of the eyeless head.

We have never failed in the many heads studied to identify in the totally eyeless half both median and inner ganglia. Plate III., Fig. 3, shows no eye on the left side, but here the section does not go through the optic tract. However, both the median and inner ganglia appear in other sections of this head. Fig. 5 shows also a totally eyeless condition on the left side and there is no trace of the outer ganglion. The section goes through the whole optic tract of this side showing clearly both median and inner ganglia, each having a double lens-like appearance as on the opposite side, where the eye is present. The left side of the head is correspondingly smaller than the right giving it a twisted appearance, and there

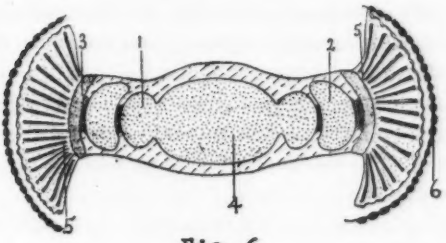


Fig. 6

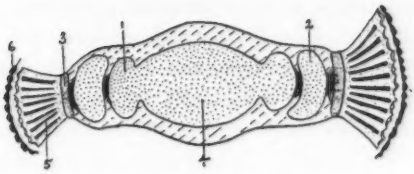


Fig. 7

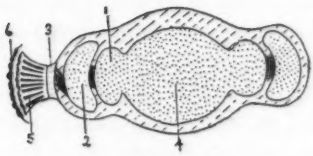


Fig. 8

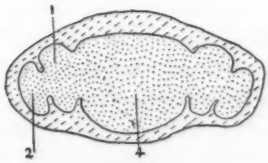


Fig. 9

FIGS. 6–9. Diagrammatic cross sections. FIG. 6, normal; FIG. 7, both eyes reduced; FIG. 8, left eye reduced, right wanting; FIG. 9, totally eyeless.

1, inner ganglion: 2, middle ganglion: 3, outer ganglion; 4, brain; 5, ommatidia; 6, facets.

is considerable difference in the size of the ganglia of the two sides. Fig. 6 shows a totally eyeless condition on the left side but here the section does not go through the optic tract. In Fig. 7 there is no trace of the eye on the left side, but the inner ganglion is shown here and the median in other sections. Figure 8 is a frontal section of a totally eyeless head with no sign of ommatidia on either side.

Text-figures 6 to 9 illustrate in diagrammatic fashion the condition of the optic tract in normal and eyeless flies. In Fig. 6 the eyes are normal and all three ganglia are present. In Fig. 7 the eyes are reduced but the three ganglia still remain. In Fig. 8 the left side repeats the condition of Fig. 7 but the right side is totally eyeless and only the median and inner ganglia remain. In Fig. 9 both sides are totally eyeless and in both the outer ganglion is absent.

Discussion.

It is an interesting fact that the loss of the eyes causes the loss of only the outer ganglion, the two inner remaining, although in a modified condition. From its structure and form we would hardly expect the outer ganglion to remain when the eye is gone. It is so intimately connected to the eye throughout its whole distal surface by fibers which pass through the basement membrane that it is difficult to see how the eye could disappear without it. The other two ganglia have obviously a very different structure. Between either two of these three ganglia the separation might easily occur, but it is evident from the preparations that the break in the optic tract has taken place at the first point where a separation is structurally simple.

There has been considerable discussion in the past concerning the homology of the optic tract of insects. Berger first pointed out the comparison between the narrow constriction separating the brain and inner ganglion and the entire optic nerve of other forms. The retinular layer he regarded as homologous only to the rod and cone layer of the vertebrate eye.

Patten however regarded the cone cells as the percipient element and thought them connected to the rhabdome. Lowne conceived of the outer ganglion as the true retina and thought that no fibers pass from the retina through the basement

membrane to the eye, but that the fibers ended in the outer ganglion; these conclusions seem not to have been confirmed by other workers. Most modern investigators agree with Parker, who has identified the rhabdomeres as the retinal elements, and has been able experimentally to produce upright images in them. His experimental conclusions are borne out by his detailed work (Crustacea) on the structure of the rhabdomeres as well as by that of Hesse. The latter has been able in insects to trace nerve fibrils passing from the rhabdomeres to the retinulæ, each retinula ending in a nerve fiber which goes through the basement membrane.

Since the rhabdome layer is to be regarded as the true retina it would seem that the optic nerve should properly be considered as distal to the ganglia rather than as between inner ganglion and brain according to Berger's conception. This is the view taken by Wheeler who regarded the optic nerve in ants as peripheral to the optic ganglion. The present work would seem to lend some support to the idea that the mass of decussating fibers between the outer and middle ganglia is the optic nerve, for when the eye disappears, it would be most natural to expect the break in the optic tract to take place at the optic nerve. It must be pointed out that we lack definite embryological information as to the origin of the outer ganglion, and until this is obtained, the suggestion just made can be only provisional.

Summary.

Flies of the mutation eyeless (*Drosophila melanogaster*) have small eyes on both sides or are sometimes totally eyeless on either or both sides. The eye and the optic tract in normal and eyeless stocks were studied in order to determinate what part of the optic tract is lacking in the eyeless flies. In the normal fly, three ganglia, outer, median and inner, connect the eye with the brain. In flies with small eyes all three ganglia are present and the ommatidia are normal although greatly reduced in number. In totally eyeless flies the outer ganglion is missing and the median and inner are contracted into a more or less shapeless mass which nevertheless discloses its double nature.

Since the retina has been identified by modern workers as the rhabdome layer of the eye, these observations lend support to the

idea that the optic nerve is a mass of fibers connecting the outer and median ganglia.

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DESCRIPTION OF FIGURES.

All drawings in this paper are by Esther Y. Furrow.

Abbreviations.

b—bristle.
bm—basement membrane.
c—cell layer.
df—decussating fibers between outer and median ganglia.
f—facet.
fb—parallel groups of nerve fibers.
ig—inner ganglion.
mg—median ganglion.
ng—nerve fibers reaching to basement membrane.
np—nucleus of pseudocone.
nr—nucleus of retinula.
og—outer ganglion.
om—ommatidium.
p—pseudocone.
r—retinula cell.
rb—rhabdomere.
rl—layer of retinulæ and rhabdomeres.
t—cut ends of trachæ.

PLATE I.

FIG. 1. Section through the head of a normal fly. × 180 (16 mm. obj. 12.5 ocular).

FIG. 2. Longitudinal section through several ommatidia of a normal fly (1.9 obj.).

FIG. 3. Cross section of a single ommatidium of a normal fly near the distal end of the rhabdomeres. × 1500 (2 mm. obj., 12.5 ocular).

FIG. 4. Cross section of a single ommatidium of a normal fly more proximal than that of Fig. 3 (same magnification as that of Fig. 3.).

FIG. 5. Details of the outer ganglia (Transverse section of head). × 825 (1.9 obj., 7.5 ocular).

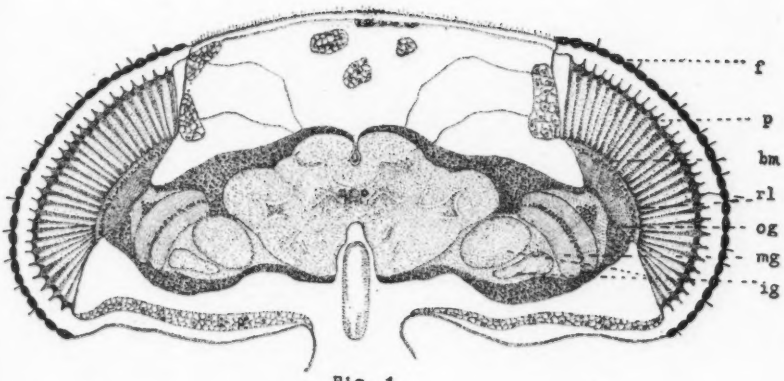


Fig. 1

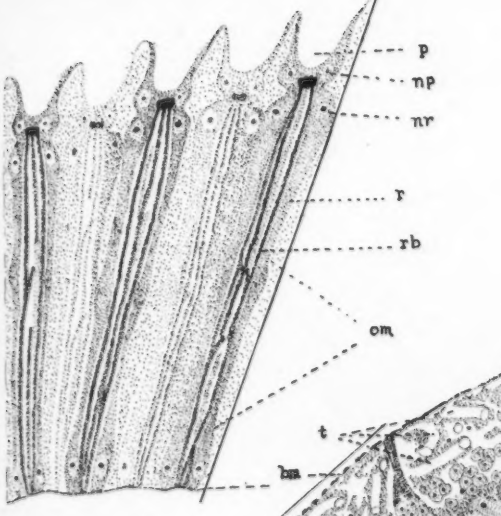


Fig. 2

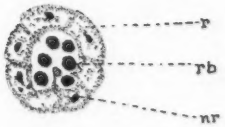


Fig. 3

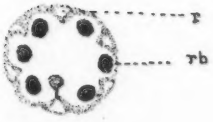


Fig. 4

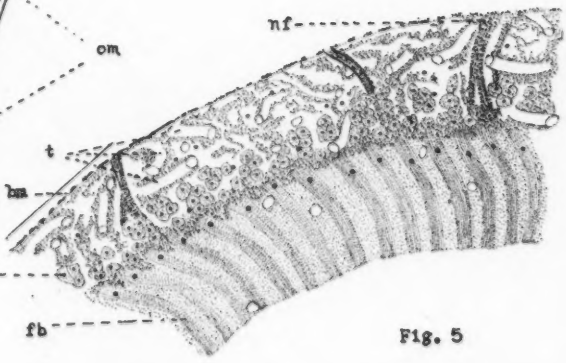


Fig. 5

PLATE II.

FIG. 1. Details of the three ganglia (Transverse section of head). × 495 (4 mm. obj., 10 ocular).

FIG. 2. Transverse section of head of fly with small eyes. Same magnification as Plate 1, Fig. 1.

FIG. 3. Transverse section of totally eyeless fly. Same magnification as Fig. 2.

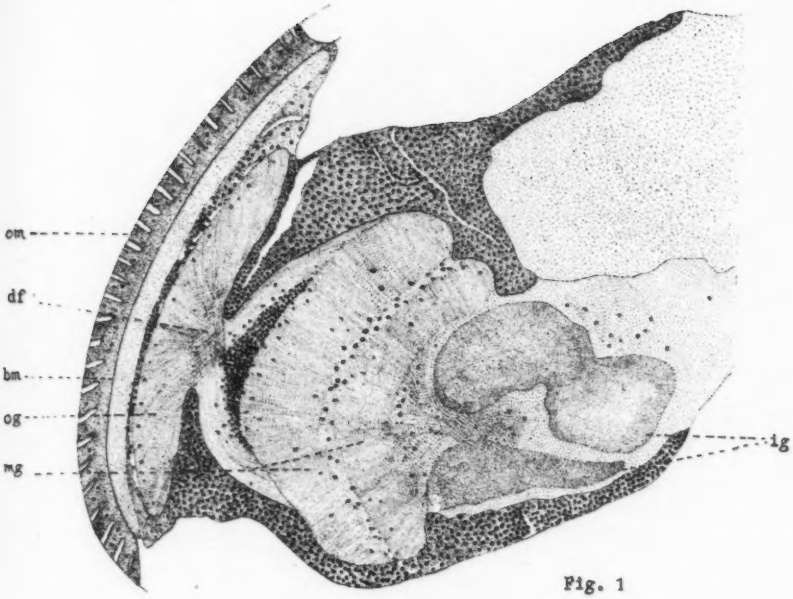


Fig. 1

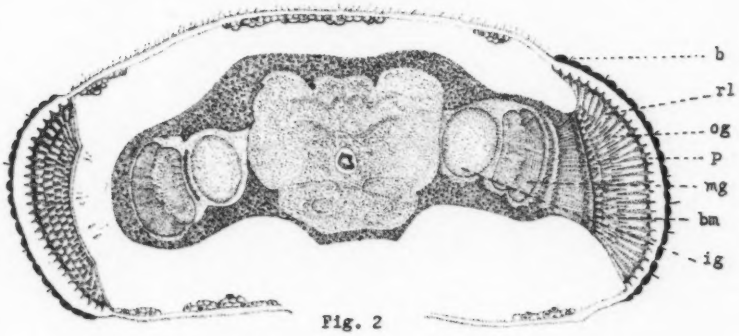


Fig. 2

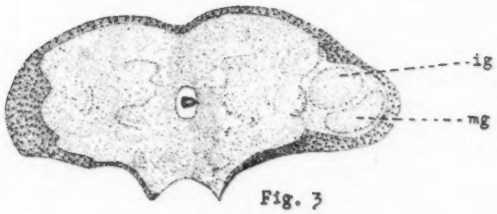


Fig. 3

PLATE III.

Microphotographs.

FIG. 1. Side view of head of living normal fly.

FIG. 2. Transverse section of head of normal fly, from slide loaned by Dr. F. Payne.

FIG. 3–7. Transverse sections of heads from eyeless stock.

FIG. 3. Head is totally eyeless on left side, has very small eye on right. Section does not go through optic tract of eyeless side.

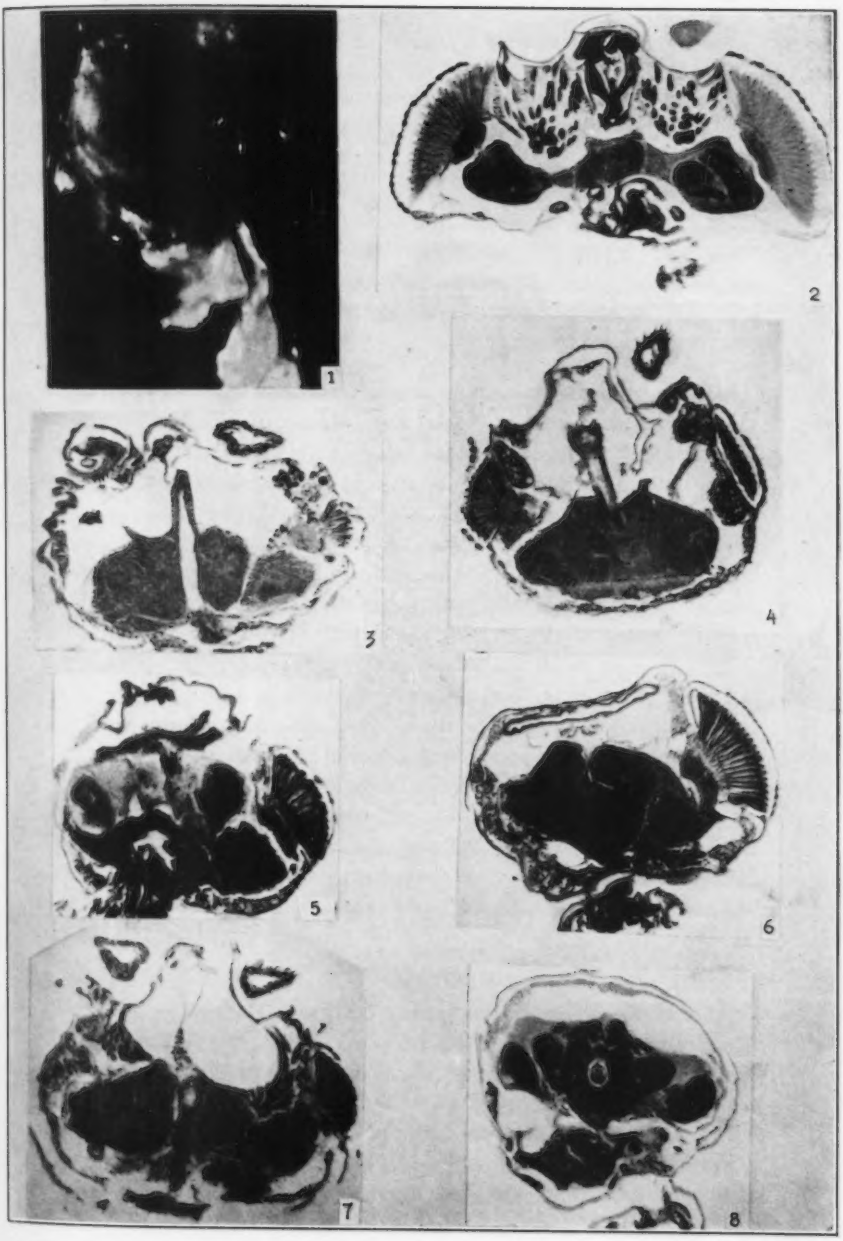
FIG. 4. Head has two small eyes. Section goes through optic tract of left side.

FIG. 5. Head has slightly larger eye on right; is totally eyeless on left. Optic tract of both sides in section.

FIG. 6. Same as Fig. 3, but eye on right side is fairly large.

FIG. 7. Head is totally eyeless on left, where section goes through the inner ganglion, but misses the median. Right side has small eye.

FIG. 8. Frontal section of head totally eyeless on both sides.



RICHARDS AND FURROW.

A RECONNAISSANCE OF THE RELATION BETWEEN DESICCATION AND CARBON DIOXIDE PRODUCTION IN ANIMALS.

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HULL ZOÖLOGICAL LABORATORY, THE UNIVERSITY OF CHICAGO AND THE BIOLOGICAL LABORATORY OF THE UNIVERSITY OF ARIZONA.

I. INTRODUCTION.

A fairly comprehensive review of the literature on the desiccation of animals reveals work largely of two general types, (1) the effects of desiccation on the behavior of the animal as shown by Tower (1906), Hennings (1907), Jacobs (1909), Breitenbecker (1911), Shelford (1913, 1914a), Chenoweth (1917), Wesse (1917), Hamilton (1917), and Bodine (1923), and (2) the water content and the vital limits of desiccation of animals as determined by Leeuwenhoek (1701), Baker (1764), Doyére (1842), Semper (1881), Durig (1901), Babcock (1912), Schmidt (1918), Bodine (1921), and Hall (1922). From the standpoint of possible physiological changes resulting from water losses in animals, little has been done and it is the purpose of this paper to point out certain definite relations between desiccation and the physiological states of a limited number of species, as determined by the excretion of carbon dioxide.

The work has been made possible largely through the kind suggestions and helpful comments of Dr. W. C. Allee. Thanks are also due Dr. L. H. Hyman for assistance in the collection and identification of material.

II. MATERIAL.

Many animals were tried and rejected as unsuitable because of the inability to keep them quiet throughout the determinations. The material was selected so far as possible with regard to the natural habitat of the animals. The meal worm, larva of *Tenebrio molitor* Linn., a xerophilous animal, the larva of the eight-spotted forester moth, *Alypia octomaculata* Fabricius, a mesohygrophilous animal, and the slug, *Agriolimax campestris*

Binney, the slimy salamander, *Plethodon glutinosus* Green, the cricket frog, *Acris gryllus* Le Conte, all hygrophilous animals. The natural environments of these animals give a fairly wide range of maximum evaporation. With the exception of *Acris* and *Tenebrio*, the animals were kept in the laboratory only a few days and in all cases were under as nearly natural conditions as possible.

III. Methods of Desiccation and Carbon Dioxide Determination.

The desiccating apparatus consisted of a series of washing bottles so arranged that air could be drawn through sulphuric acid, granulated zinc, and glass wool into the desiccating chamber. The air from the desiccator was bubbled through a methyl orange solution for three hours to test for sulphuric acid. This test was repeated at intervals throughout the work and at no time were there indications of sulphuric acid in the air.

The respiratory apparatus devised by Osterhout was used to make the carbon dioxide determinations. Since this apparatus has already been described (Osterhout, 1918), a brief summary will suffice: the apparatus consists of a closed system in which air is circulated by means of a pump. There are two possible ways for the air to pass from the animal chamber, either directly into a Pyrex glass tube containing an indicator solution, or indirectly through a container of sodium hydroxide which removes the carbon dioxide from the air before it reaches the indicator solution. Thus, the desired color may be restored to the latter without the necessity of a change of indicator. This is highly advantageous because a series of determinations is possible without the admission of air to the closed system. In all experiments, 4 cc. of an aqueous solution of phenolsulfonephthalein were used. The indicator solution was made at the beginning of each experiment in sufficient quantity to last throughout one complete experiment, except when the experiment ran for several days, which occurred only in the work on the larvæ of *Tenebrio* and *Alypia*. A north skylight lamp and a white background made of absorbent cotton were employed to avoid the changeable color quality of daylight and furnish a constant light for work at night upon the

critical comparisons of the known and unknown indicator solutions.

Wherever the respiration time of animals appears in this paper, it is to be taken as the time in seconds required by the animal, or animals, to excrete sufficient carbon dioxide to change 4 cc. of standard indicator solution from pH 7.8 to pH 7.2. No attempt was made to determine the actual quantities of carbon dioxide produced by the animals, since all comparisons were made on the basis of time required for the excretion of sufficient carbon dioxide to produce this standard change in pH value.

The control of the movements of the various animals in the respiratory chamber was on the basis of tropistic responses to light or contact. In no instance was cessation of activity produced through mechanical means, and no experiments have been recorded in this paper where the animal was moving when the carbon dioxide determinations were made. Movements could easily be detected through marked irregularities in respiration time.

Controls were run on all experiments. The control animals were kept under the same conditions as the experimental animals except they were not desiccated and exclusive of one control in the experiments on *Tenebrio*, neither controls nor experimental animals were fed during the experiment.

The animals were weighed in previously adjusted weighing bottles immediately after the carbon dioxide determinations were made. This proved preferable to weighing before the determinations were made since it reduced the stimulating effect of handling and consequently the animals came to rest in the respiratory apparatus more quickly. The animals undoubtedly lost weight in the animal chamber of the respiratory apparatus in some instances and gained weight in other instances, depending upon the state of desiccation, but the gain or loss in the respiratory chamber was so slight compared with the loss in the desiccator that it was disregarded. All weights are given in milligrams as a matter of convenience.

The general procedure was the same for all of the experiments conducted. The normal respiration time of the experimental animal was determined; three consecutive readings were averaged for each determination. These readings varied somewhat

because of the impossibility to detect always the exact end-points; in some instances the variation was as much as 2 per cent and throughout the work the variation was greater with the shorter respiration periods than with the longer. The animal was then weighed and placed immediately into the respiration chamber of the desiccator. The normal respiration time of the control was determined and its weight recorded while the experimental animal was being desiccated. This was repeated at intervals, usually until the experimental animal showed no signs of life. The intention was to obtain the rates of carbon dioxide excretion at approximate stages of from 5 to 10 per cent. loss in body weight. The time necessary for a loss in body weight of about 10 per cent. was previously determined with animals of similar weight.

IV. Experimental Results.[1]

A. Xerophilous Animals.

Meal Worm, Larva of Tenebrio molitor Linn.—These worms were kept in the laboratory in open Mason jars partially filled with air dried wheat bran. Five series of experiments were performed. Three worms were used in each experiment to reduce the respiration time. The controls of four of the five series were not fed. The controls of the fifth series were fed throughout the experiment. The first readings were the most difficult to obtain on account of the activity of the animals. A dark chamber was the only means used to keep the animals quiet, although it was found that animals placed with their bodies in contact with both the bottom and side of the animal chamber not only remained quiet but also were less inclined to aggregate. After 10 hours in the desiccator the meal worms were inactive when undisturbed. In the first four series, carbon dioxide determinations were made regularly at 24-hour intervals for six days. In the fifth series determinations were made at shorter intervals. Evaporation from the meal worm is very slow even in dry air. Hall (1922) found that it required 1,084 hours to desiccate a meal worm to 52.6 per cent. of its body weight. While this loss was a greater percentage of body weight than the average percentage of water contained in the body, the worm lived two days after desiccation.

[1] Complete experimental data are on file in the University of Chicago Libraries.

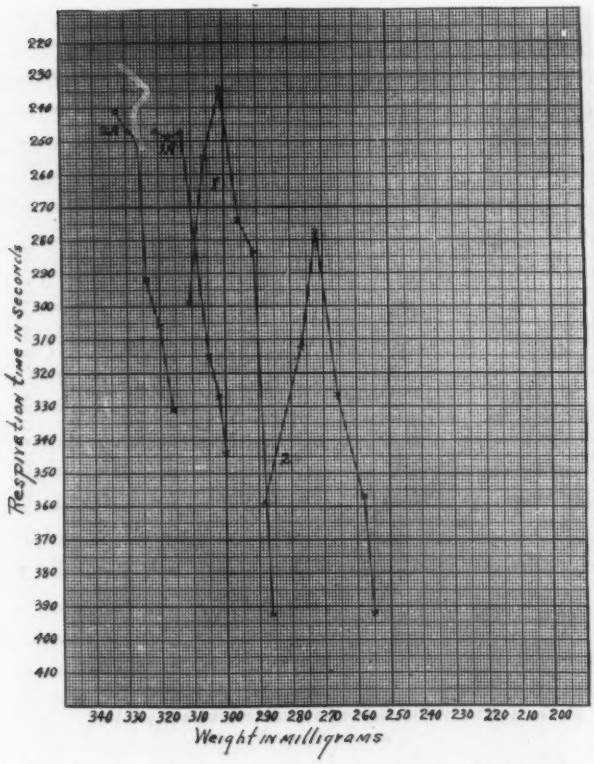

FIG. 1. The effect of desiccation on the carbon dioxide production of the meal worm. Points (*x*) denote determinations at 24-hour intervals. Curves 1*A* and 2*A*, controls of 1 and 2 respectively. Note marked decrease in carbon dioxide output at 72-hour determinations of both experimental animals and controls.

B. Mesohygrophilous Animals.

Larva of the Eight-spotted Forester Moth, Alypia octomaculata Fabricius.—This larva was taken from the Boston ivy (*Ampelopsis*), which grew on the walls of the laboratory, and placed directly into the animal chamber of the respiratory apparatus. Preliminary experiments proved the animal to be very active and stimulated by contact with the glass wall of the chamber. Fairly satisfactory results were obtained by placing a rough piece of wood, which had been oven dried and tested for carbon dioxide, on the bottom of the chamber upon which the animal became

quiet when the chamber was darkened. This piece of wood was kept in the animal chamber throughout the experiment in order that the reduction in the volume of the apparatus would remain constant.

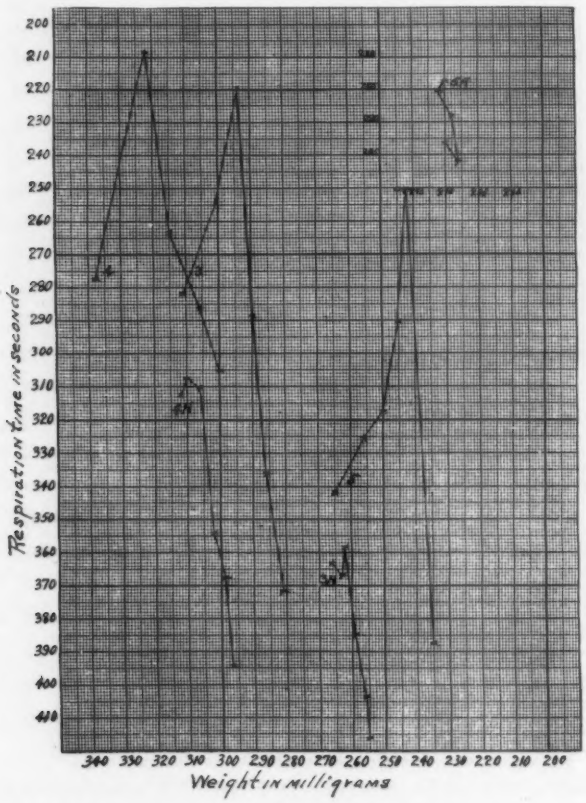

FIG. 2. The effect of desiccation on the carbon dioxide production of the meal worm. Experiments 3 and 4 with controls 3*A* and 4*A* on animals not fed during experiments and with determinations at 24-hour intervals. In experiment 5 the control animals, curve 5*A* (twice experimental scale) were fed. Point (o–x), curve 5, denotes carbon dioxide determination after 68 hours of desiccation.

Both the experimental animal and control lost about 55 per cent. of the body weight in 70 hours. The animals were still active but much reduced in size. The experimental animal and

control showed a decrease in the rate of carbon dioxide production, the experimental animal from the first desiccation, and the control after 8 hours. The available data are insufficient to permit of any definite conclusions. However, two possibilities

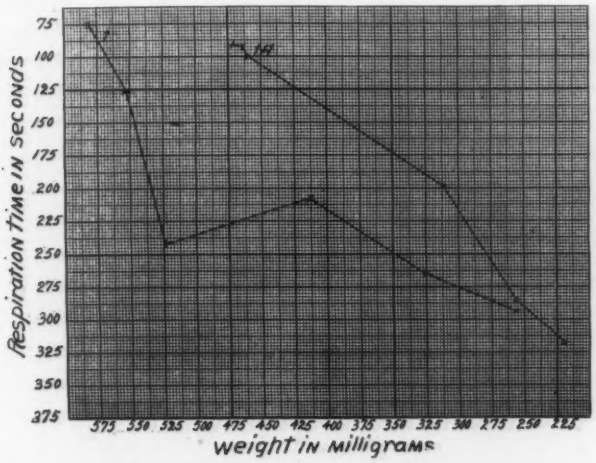

FIG. 3. The effect of desiccation on the carbon dioxide production of the larva of the eight-spotted forester moth. The time intervals of determinations (x) were the same for the experiment, curve 1, and control, curve 1A; first, 3 hours, second, 5 hours, third, 24 hours, fourth, 18 hours and fifth 14 hours.

are suggested by this and several preliminary experiments: one, the partial closure of the spiracles to retard the loss of water, and the other, the effects of starvation. Similar results were obtained in trial experiments with the larvæ of two other species of Lepidoptera (unidentified) and the centipede, *Scolopendra heros*.

C. Hygrophilous Animals.

Slug, Agriolimax campestris Binney.—The experimental animals were taken from their normal environment in a greenhouse and placed in the animal chamber of the respiratory apparatus. The control animals were kept in moist filter paper in a closed dark chamber where the rate of evaporation and temperature were approximately that of the normal environment. Single individuals were used in each of the four series of experiments recorded. The animal chamber of the respiratory apparatus was

covered with black paper and a small opening at one side permitted of observation without disturbing the animal by the movements involved in the manipulation of the apparatus. The slugs usually came to rest in a short time in the darker region of the chamber. Series of determinations were made until the readings became constant.

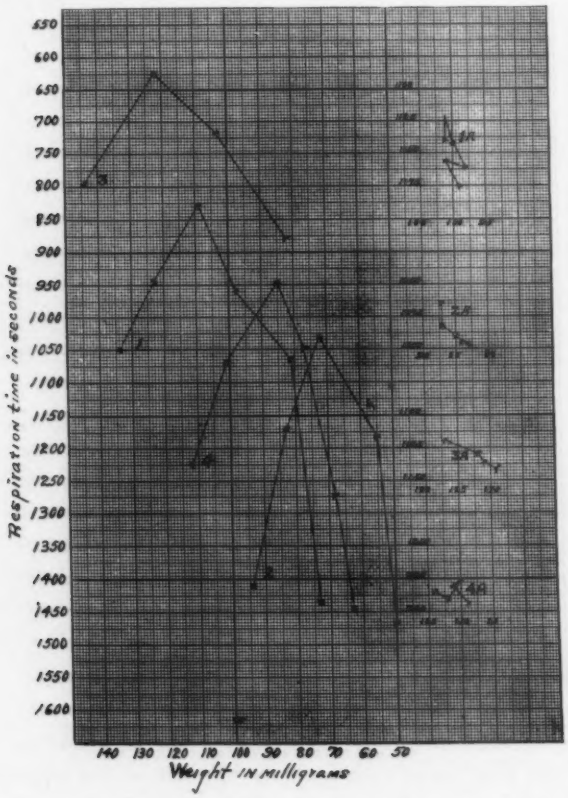

FIG. 4. The effect of desiccation on the carbon dioxide production of the slug. Control curves indicated by letter (*A*) and enlarged to twice the scale of experimental curves. Total time of each experiment about 8 hours.

The rate of carbon dioxide production increased with desiccation until there was a loss of water equivalent to from 20 to 30 per cent of the body weight when the rate fell below normal and

continued to decrease with further loss of water. The time between the first and last determinations was too short to permit the starvation factor to enter into the results. The controls lost some water in the duration of a series, a period of about 8 hours. One control lost 6.29 per cent. of the body weight, the greatest loss of any control, while the experimental animal of the same series lost 43.05 per cent. of the body weight. The respiration rates of the controls seemed to be little, if at all, affected by the slight losses of water.

Salamander, Plethodon glutinosus Green.—The salamanders gave up water very quickly. Results show that 10 per cent. of their body weight was lost in 15 minutes of desiccation. The greatest loss of the four individuals experimented upon was 42.92 per cent. of the body weight, distributed over a period of 8 hours.

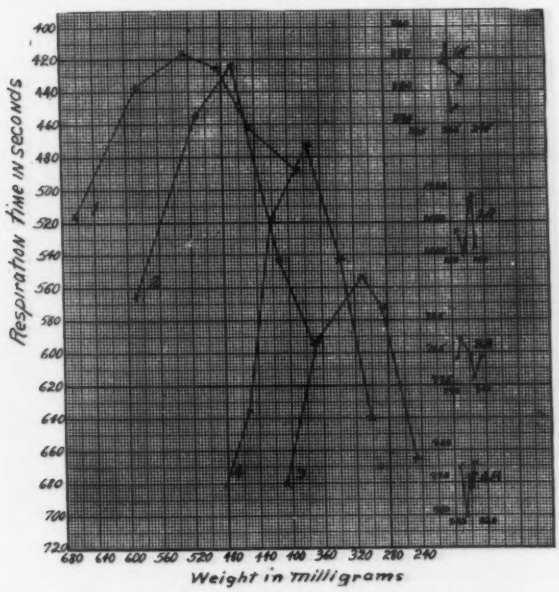

FIG. 5. The effect of desiccation on the carbon dioxide production of the salamander. Control curves indicated by letter (*A*); numbers correspond to the experimental curves. Control curves twice experimental scale.

This individual lived 6 hours after the last desiccation. Hall (1922) desiccated *Ambystoma punctatum* to 47 per cent. of their

body weight without loss of vitality. The salamanders proved easy to handle, oriented themselves negatively to light in the darker region of the animal chamber and remained quiet. When placed in the desiccator for the first time, the animals were quite active for 5 minutes, never longer. Carbon dioxide determinations prior to losses of 20 to 25 per cent. in body weight showed a rapid increase while continued desiccation resulted in a decrease in carbon dioxide output. The control animals lost very little water, not enough to appreciably affect the rate of carbon dioxide production.

Frog, Acris gryllus Le Conte.—Adult frogs of this species which

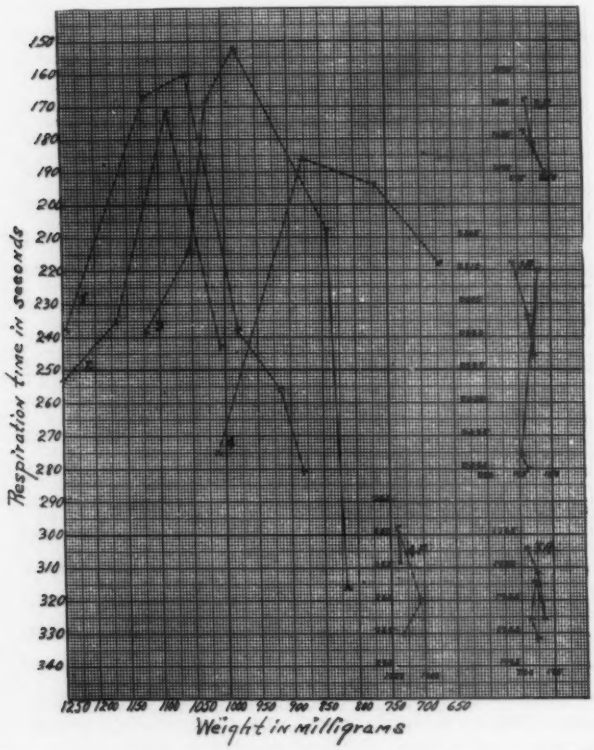

FIG. 6. The effect of desiccation on the carbon dioxide production of the frog. Points (x) on control curves (indicated by letter *A*) correspond to determinations on experimental curves. Control curves twice experimental scale.

had been kept in the laboratory for several days were used. To quiet the animals proved rather difficult. A dark chamber with a small opening in the paper covering caused the animals to orient themselves towards the light. After being handled the respiratory rates of the animals returned to normal very slowly, that is, a number of consecutive readings showed a decrease in rate before they became constant. This suggested the possibility of oxygen depletion within the closed system. Krogh (1916) in summarizing the work of several authors concluded: "In cold blooded animals oxygen consumption is practically independent of the oxygen pressure down to about 2 per cent. pressure." Fresh air was admitted to the animal chambers without disturbing the animal, by the removal of the indicator tube. This had no effect upon the rate of carbon dioxide production, so it was assumed that the animal had obtained sufficient oxygen. The rate of carbon dioxide production in the desiccated frogs of the four series of experiments increased with loss in weight until the loss amounted to from 15 to 20 per cent. of their body weight. The rate diminished subsequently to the completion of the experiment. The total time of a single experiment was less than 14 hours and hardly permitted of starvation as a factor in the decrease in respiration rate. The control animals lost very little water, from 0.26 to 1.47 per cent. The respiratory rates of the control frogs were fairly constant throughout the work.

V. Discussion.

These investigations show that desiccation stimulates the physiological activity of certain animals and that the increase in irritability is followed by marked depression. While there is no literature on the effects of desiccation on the carbon dioxide output of animals, it is interesting to note that Bodine (1922) obtained somewhat similar results from the action of ether, xylol and acetone upon grasshoppers, and concluded that anesthetics have physiological effects other than on respiration. Shelford (1913), with behavior methods, found that animals subjected to air of low humidity were more active than normal ones, and if kept under such conditions, the period of activity was followed by a period of depression, and suggested that the increased irritability was probably due to the concentration of the blood and

tissue fluids. Since the present results are based on physiological determinations, they are hardly comparable with results from behavior studies, however, it seems reasonable to suppose that if this were the entire cause of the stimulation, the increased irritability would continue, with additional losses of water, to a point nearer the vital limit of desiccation than has been found in the present experiments. Also, Shelford refers to irritability as evidenced by the activity of the animals, while these experiments show that the increased metabolic activity continues long after the animals become inactive. To cite a special case, the meal worms were active for the first 6 to 10 hours in the desiccator but the respiratory rate increased for more than 60 hours.

Since meal worms with a water loss of less than 1 per cent. of the body weight gave an increase in respiratory rate of more than 10 per cent., increased concentration of body fluids could hardly be the principal factor in causing the increase and suggests that this is, in part, a function of the nervous system. The truth of this assumption is open to investigation and further work will be done along this line.

There is a rough correlation between the highest respiratory rates and the vital limits of desiccation. According to Hall (1922), the vital limit of the frog is 41 per cent. and that of the salamander 47 per cent. The highest respiratory rate of the frog, from an average of four experiments, corresponded to 13.66 per cent. loss of body weight and with the salamander to 21.87 per cent. loss of body weight. The xerophilous animals gave up water very slowly and in proportion to water losses were stimulated more than the hygrophilous animals.

The decrease in carbon dioxide elimination with further desiccation is even more difficult to explain. No literature on studies pertaining to either starvation or desiccation of the larvæ of *Tenebrio* and *Alypia* has been found, but Hill (1911) showed that the heat production of the frog decreased with starvation and Child (1919), Hyman (1919) and Allen (1919) who worked with *Planaria*, and Bodine (1921) with grasshoppers, all pointed out that starvation decreased the rate of carbon dioxide production. The results from the meal worm experiments indicate a stimulation from desiccation until the depression of food starvation became a greater factor. Particularly, since the respiratory

rates of both the experimental animals and controls decreased after 48 hours of desiccation without food, while the fed controls showed only slight variations. Water starvation undoubtedly increases the depression of food starvation, but this fails to explain the decrease in respiratory rates of animals not affected by starvation.

In work on pithed frogs, the author has observed that handling often produced reflex movements, but after the frogs had lost considerable water these reflex movements did not occur, and furthermore, were not induced when the sides of the body or feet were pinched. This suggests the possibility that after the nerves have lost a certain percentage of water, the passage of the nervous impulse is retarded or inhibited. If this be true, in part or whole, the highest respiratory rate should immediately precede the disappearance of reflex movements, but no data are available on this point. Durig (1901) found that the nerves of a frog transmitted more slowly and the latent period of the muscles increased with water losses from 9 to 30 per cent. of the body weight, so that the decrease in respiratory rate is what might be expected if this were true.

Until more data are available, it is difficult to suggest the causal factors, but the effect will probably be found to be in part caused by accumulation of waste products due to poor elimination and also to inadequate distribution of food and oxygen to the cells. It is also highly probable that the decrease in water content has a direct action in slowing down certain of the oxidative processes. Decreased metabolism from any or all of these causes would appear in the experiments as conducted.

VI. Summary.

1. Desiccation is followed by marked changes in the physiological states of certain animals.

2. *Agriolimax campestris* Binney, *Plethodon glutinosus* Green, *Acris gryllus* Le Conte, and larvæ of *Tenebrio molitor* Linn show a definite, positive, correlation between desiccation and carbon dioxide production.

3. The above-named animals show a gradual increase in carbon dioxide production when desiccated to less than one half of the possible vital limit. Continued desiccation is followed by a

decrease in carbon dioxide production, which falls below normal before the vital limit is reached.

4. The larvæ of *Alypia octomaculata* Fabricius show a decrease in carbon dioxide production in all stages of desiccation.

5. Xerophilous animals give up water more slowly and in proportion to water losses are more stimulated by dry air than hygrophilous animals.

6. Water starvation increases the depression of metabolic activity caused by food starvation.

7. Extremely dry air is a protoplasmic irritant.

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EXTERNAL PARASITES OF BIRDS AND THE FAUNA OF BIRDS' NESTS.[1]

IRENE D. DOBROSCKY.

There have been comparatively few papers published on the parasites of birds. The work of the writer on this subject was carried on at Cornell University with material collected in the vicinity of Ithaca, New York.

The external parasites of the birds examined were all Arthropods falling into three orders,—Diptera, Mallophaga, and Siphonaptera. The dipterous parasites belong to the genus *Protocailiphora* of the Family Calliphoridæ. The majority of Calliphorine flies are scavengers that live on decaying animal and vegetable matter. The genus *Protocalliphora*, however, is a small group of flies parasitic on young birds. The larvæ are intermittent blood-sucking obligate parasites. There is some controversy as to whether they are confirmed ectoparasites or not. Bezzi ('22) states that the subcutaneous tumors caused by fly-larvæ are in many cases erroneously attributed to the *Protocalliphora* and that other Calliphorine flies may be the agents. Since the *Protocalliphora* are intermittent feeders it is impossible for them to become permanent ecto parasites.

The nomeclature of this group is somewhat involved. In earlier literature the genus *Protocalliphora* is referred to under the names of *Phormia* and *Protophormia*. *Avihospita* is a later synonym. There are at least five *valid* world species in this genus. In Europe there are two distinct species—*P. azurea* Fallen and *P. cærulea* Robineau-Desvoidy. According to the studies of the writer based on larval and pupal forms, and on adults by R. C. Shannon, there are at least three distinct species in North America. These are *P. splendida* Macquart, *P. avium*, and *P. hirudo* (a Western form), the latter two described by Shannon and Dobroscky ('24).

[1] The writer wishes to express her appreciation for help, suggestions, cooperation and determinations to Dr. R. Matheson, Dr. O. A. Johannsen, Dr. J. Bequaert, Dr. W. T. M. Forbes, Professor G. W. Herrick, C. R. Crosby, R. C. Shannon, J. W. Folsom, F. C. Fletcher, H. Good, C. H. Curran, L. S. West, J. R. Mallock, H. C. Huckett, and to the numerous persons who aided in collecting the birds' nests.

The writer's work upon parasitic larvæ has been confined to *P. avium*. This larva, which is a parasite of the crow, is amply described and figured by Coutant ('15) under the name of *P. azurea*. The larval period is from ten to thirteen days, and consists of three stages. Larvæ measuring from 2 mm. to 15 mm. were found in the nests of crows. If a third stage larva has not attained full size, and cannot obtain food, it will pupate, and the emerging adult will be smaller than usual.

Pupal cases belonging to *P. avium* and *P. splendida* collected from the nests of several species of birds were carefully studied. Within each species differences in the pupal cases of the parasites from different bird hosts were observed. Since the adult flies also show distinct differences, according to the host, it seems probable that the variations are due to heredity, with high specificity, and are not due to the fact that the larvæ have fed upon different hosts. The most noticeable variation in the pupal cases was in the size and arrangement of spines around the stigmal area. The spines in the parasite on the house wrens were .05 mm. long, while in the puparia found in the nest of the scarlet tanager they were so minute that they seemed like mere stipples. In some the spines are inserted directly into the underlying chitin and in others they are on raised ridges. A more detailed description of these host to host variations, with illustrations, is given in the writer's thesis on this subject on file at Cornell University.

The method of hibernation of this parasite is not known. It seems most probable that it winters over as an adult. Since the eggs must be laid in new nests in order to hatch in the immediate presence of young nestlings, the egg stage is ruled out as a method of hibernation. Of the hundreds of pupal cases collected during the fall, winter, and spring months none were alive. They were either empty, or dead and dried up, or parasitized by a small hymenopteron, of which note will be made later.

The bird hosts of the North American species of the genus *Protocalliphora* are as follows:

I. Those observed by the writer: Robin, bluebird, house wren, crow, scarlet tanager, indigo bunting, chipping sparrow, song sparrow, catbird.

II. In addition those listed in the joint paper by R. C. Shannon

and the writer based on material in the U. S. National Museum: Brown thrasher, cardinal, cooper's hawk, long-eared owl, cliff swallow, western robin, western horned-lark.

III. In addition those listed from California by O. E. Plath: Yellow warbler, green-backed goldfinch, willow goldfinch, Nuttall sparrow, California purple finch, California linnet, California brown towhee, rusty song sparrow, Oregon towhee, russet-backed thrush, cedar waxwing.

IV. In addition those recorded by Bezzi: Bank swallow, swamp sparrow, white-throated sparrow, white-crowned sparrow.

V. Henshaw ('08) records: Woodpeckers.

Since these parasites are so prevalent, no doubt in the course of further investigation, many more species of birds will be found to be parasitized.

Economic Importance.

The results of the writer's collecting show the following amount of infestation:

Host.	Nests Examined.	Nests Infested.	Deaths.
Robin	202	28	None.
Crow	17	7	None.
Bluebird	5	5	Three.
House wren	26	9	Two.
Indigo bunting	2	1	None.
Scarlet tanager	1	1	None.
Catbird	15	1	None.
Chipping sparrow	31	1	None.
Song sparrow	20	1	None.

The number of pupæ in a nest varied. In robins' nests there were from 1 to 20 pupæ; from 10 to 50 were found in the bird-houses of house wrens and bluebirds. Other nests, except those of the crow, contained usually from 1 to 4 pupæ. The crows' nests however, contained anywhere from 20 to 343. The nest in which the 343 larvæ of all sizes, were found, harbored three young crows. These were much smaller and weaker than crows of their age usually are.

A parasite with such a wide range of valuable birds as hosts

must cause considerable harm. The problem, therefore, presents a serious economic aspect. Henshaw ('08) states that this parasite on bluebirds produces almost 100 per cent. mortality. Plath in California found a mortality among nestling birds of 5 per cent. to 10 per cent. Coutant and Dufour report no mortality among crows and swallows. The experiments of the writer indicated no mortality, but young crows from parasitized nests showed a marked retardation of growth, and young robins became so weakened that a slight exposure to cold was sufficient to cause their death. However, in collecting, the parasitized nests of bluebirds and house wrens were found to contain the bones of nestlings. The presence of the parasites seems to indicate that they were responsible for the deaths. No doubt in the natural habitats the presence of these parasites weakens the young birds and indirectly causes their death by lowering their resistance to unfavorable conditions and causing them to fall an easy prey to other animals.

Mallophaga, or bird-lice, are parasites which have accompanied the whole phyletic history of birds. Harrison ('14) who has written a very interesting paper on this subject says, "I would suggest that the adoption of a parasitic habit by mallophagous insects occurred even as far back as the late Mesozoic time." In their primitive form he says they parasitized both birds and marsupials before the true mammals differentiated out. H. E. Ewing identified some bird lice collected by the writer from a crow as *Myrsidea subæqualis* Lay.

One does not usually think of fleas as parasites on birds, but they are to be found quite frequently in bird nests. Numerous fleas were collected from the nests of bluebirds, house wrens, bank swallows, and house sparrows. Adult fleas, in copulation, were taken from the nests of bluebirds, house wrens, and house sparrows which were collected in the month of January. A bank swallow's nest examined in June contained enormous numbers of larvæ. Posted at the lower part of the hole which is the entrance to the inhabited nests, are the adult fleas. They probably attach themselves to the parent birds and are so spread throughout the colony. All these fleas noted above were determined by R. C. Shannon as belonging to the genus *Ceratophyllus*.

The Fauna of Birds' Nests.

Besides the inhabitants that parasitize birds, there were found a great variety of other insects and animals of lower orders. They use the nests as a place to hibernate or to breed; in the latter case the larvæ feed on the animal and vegetable debris in the bottom of the nest. These commensals are namely sow-bugs, spiders and mites, and numerous species of insects.

The common sow-bug was encountered several times in the nests of robins. As these nests are usually damp it is not surprising that this animal should be found in them.

Spiders of several species are found in almost all bird nests, especially during the winter months. The majority of the spiders found in the nests of robins, crows, house wrens, bluebirds; chipping, song, and field sparrows, catbirds, and woodthrushes were in the immature stages. Egg-sacs were also found in the nests of bluebirds and house wrens during the winter months.

Numerous mites were found in the nests of crows, robins, house wrens and house sparrows. These were identified by H. E. Ewing as belonging to five distinct species.

Of the Collembola or spring-tails two species were encountered frequently in the nests of crows, woodthrushes, catbirds, song sparrows, red-eyed vireos, and robins. These were identified by J. W. Folsom as *Entomobrya assuata*, a new species, yellowish orange in color; and a smaller purple species, *Isotoma arborea*.

A species of Psocids, a group to which the common book-louse belongs, was found in nests of robins and house sparrows in appreciable numbers all year round.

Two species of thrips (Physopoda) occured in the nests of robins and field sparrows. *Limothrips denticornis* is an European species three records of which have been reported from New York. The writer collected this species on two occasions from bird nests and there is another record from wheat heads. The common mullein thrips, *Neoheegeria verbasci*, was found in a number of nests.

Several adult Hemiptera, or what may be truly termed "bugs," were found in the nests of robins. Where they were probably hibernating. They were leaf-eaters belonging to the Family Tingitidæ and the Family Capsidæ.

Homoptera, represented by the pear psylla and leaf-hoppers, were found in robins', catbirds', and song sparrows' nests. They were adults and were using the nests for hibernation purposes.

It is not at all uncommon to find moths in connection with bird nests. The Tineids, to which our common clothes moth belongs, are found in nests of the robin, crow, house sparrow, redstart, and chipping sparrow. Larvæ pass the winter in pupal cases made of the grasses of the nest and resemble caddis-fly cases. Adults emerge in May when there are fresh nests in which they lay their eggs, according to the statement of one European worker. The adults were identified by W. T. M. Forbes as *Tinea carnariella*. Several adults of *Carnarsia ulmiarrosorella* emerged from robins' nests in the early part of June. As the larvæ feed on shade trees, they probably use the nest as a place to hibernate.

Of Diptera or flies there were more species found than of any other order of insects. Several of them pass through their complete life cycle in the nests.

Calliope flaviceps, a small fly belonging to the Family Sapromyzidæ, is encountered frequently and in large numbers in the nests of the robin, woodthrush, redstart, song sparrow, goldfinch, catbird, red-eyed vireo and chipping sparrow. Numerous small white larvæ and flat yellow pupæ are found in the bottom of nests in the fall, winter, and spring months. The larvæ feed exclusively on the debris and seeds they find in the nests. Adults emerge early in May. It would seem that the nests of birds are the natural habitat of this species but the fact has not been noted before.

Four species of Anthomyidæ were found. Larvæ and pupæ of *Anthomyia pluvialis* were found in nests of the robin, bluebird and chipping sparrow. Adults emerged from March to May. Larvæ of *Fannia canicularis* were found in the nests of robins, bluebirds and house sparrows. Adults of this species emerged in May. *Hydrotæa nidicola* is a new (manuscript) species described by J. R. Malloch. Pupæ were found in robins' nests, and adults emerged in the latter part of April. *Hylemyia spizella* is also a new species to be published by H. C. Huckett. The flies emerged from robin and chipping sparrow nests in March.

Clausicella usitata is a small Tachinid found in the nests of

robins, crows, bluebirds, and song sparrows. As all the Tachinidæ are parasitic on other insects, this species was probably parasitizing the other Arthropods in the nests.

Three species of Sarcophagids or flesh-flies were found. *Sarcophaga sinuata* was found in the nests of the catbird, and field sparrow. Adults emerged during the month of March. *Sarcophaga spuria* and *Lacchoprosopa avium* are both new (manuscript) species described by C. H. Curran. The former was found as a pupa, and the adult emerged under laboratory conditions in March; the latter species, obtained from a crow's nest, emerged in March, also the larvæ of Sarcophagids live on decaying vegetable matter and animal refuse, and therefore, birds' nests are ideal places for them.

Small adult flies of the Oscinidæ, Phoridæ, and the Mycetophilidæ were found in crows' nests in the spring months.

Long thin dipterous larvæ with nineteen segments were found in house sparrows' nests. These were reared and proved to be *Scenopinus fenestralis* the so called window-fly. The larvæ feed on the straw and twigs of the nests.

Numerous species of beetles were found in nests. The majority were in the adult stage and seemed to be using the nests for hibernation purposes. However, in the house sparrows' nests numerous larvæ of Dermestids and Tenebrionids were found, which feed on the straw of the nests. A larva of *Malachius æneus*, an European beetle, was found in a robin's nest and reared to an adult. Some of the adult beetles found in robins' nests were: *Ptinus fur, Crioceris asparagi, Prasocuris vittata;* and Curculionid, Scymnus, Melanophthalma and Bembionid species.

The Hymenoptera found were either parasites of other insects or used the nests for hibernation. One small green hymenopteron, identified as *Mormoniella brevicornis*, was found frequently in nests which contained pupæ of *Protocalliphora*, the bird parasite referred to in this paper. These small hymenopterons parasitize the muscid pupæ, and about fifteen issue from one pupal case. Other Hymenoptera found in nests were identified as *Megastigmus nigrovariegatus*, *Passalæcus* species, *Eucoila* species, *Hyperacmus tineæ*, *Aenoplex betulæcola*, *Apanteles carpatus*, and *Tetrastichus* species. These were found as adults which were probably hibernating during the winter months.

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THE METHOD BY WHICH *TRICHONYMPHA CAMPANULA*, A PROTOZOÖN IN THE INTESTINE OF TERMITES, INGESTS SOLID PARTICLES OF WOOD FOR FOOD.

L. R. CLEVELAND.[1]

The intestinal protozoa of wood-feeding termites represent a unique and peculiarly interesting faunal association. The protozoa, very abundant both in form and number and completely filling the large and much distended gut of their host, take into their bodies and digest practically all the wood which the termites eat. The termites themselves cannot digest wood or cellulose, and hence cannot live on wood, their normal diet, without protozoa to digest it for them.

From one third to one half the body of *Trichonympha campanula*, the largest and principal wood-ingesting protozoön harbored by *Termopsis*, is filled with wood fragments, though the method employed in taking them in has been a complete mystery.

It is possible that many of the termite protozoa take in wood particles from the intestine of their host similarly to *Trichonympha campanula*. Most of them, like *T. campanula*, have no cytostomes.

Several suggestions have been made as to the possible methods of food ingestion by these protozoa. Leidy ('81), in his account of termite flagellates, called attention to the presence of food bodies and the lack of any visible channel for their entrance into the body. Kent ('84) claims to have discovered an oral aperture at one side of the body, a short distance from the anterior end. From this opening he traced a narrow œsophagus emptying into a digestive cavity in the posterior region of the body. He studied the organisms in thinly diluted milk, and says that both the pharynx and digestive tract were frequently filled with milk corpuscles. The organism which Kent observed was taken from an unknown termite of Tashmania and belonged, according to

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Kent, to the genus *Trichonympha* (*T. leidyi*), although Koidzumi ('21) has questioned (and perhaps rightly so) whether it belonged to this or another closely related genus.

Porter ('97), working with *Trichonympha agilis* from *Reticulitermes flavipes*, attempted to confirm Kent's work, both in the living animal and by means of sections of the body, but was unable to find any trace of an oral aperture. He suggests that the food particles may be drawn to the posterior part of the body by the flagella and there ingested through the thin pellicle. Concerning the possibility of the wood particles being ingested at the anterior end, Porter says: "It seems highly improbable—to say nothing of the absence of a permanent oral aperture—that solid food should pass through this region so quickly that not a single case of its presence in this part should have been discovered by any of those who have studied these parasites."

Of Porter's suggestion, Kofoid and Swezy ('19) remark: "Unfortunately the evidences for this are unconvincing." It is the opinion of these authors that in *Trichonympha campanula* the centroblepharoplast may function as a cytopharynx; yet they say, "that the centrosome should form part of the mouth structures, however, seems hardly plausible, but scarcely less so that its food should be taken in at the posterior end of the body." Cutler ('21) says, "a grave objection to this conclusion [the conclusion of Kofoid and Swezy, '19] is that food particles are never found in the anterior end of the body. One is thus drawn to the belief that food is incorporated into the body at the posterior region, though the method is still unknown."

More recently Swezy ('23) has described a pseudopodial method of food ingestion in *Leidyopsis* and *Trichonympha*. But it seems to me what she has really seen and has described is only the last stage of the unusual ingestion of very large pieces of wood, by the method described in this paper. Certainly, not one individual in ten thousand is ever seen to take in such large pieces of wood as she has figured. Instead, they feed on the smaller pieces of wood which they may ingest much more readily and which do not distend their bodies in all kinds of abnormal shapes, such as Swezy figures to be the method of food ingestion.

Perhaps the chief reason why Swezy did not see more of the process is due to the fact that she did not find a suitable medium

in which to observe the organisms while feeding. She only observed them in the intestinal fluid and in this, as anyone who has ever studied termite protozoa knows, the number of protozoa present is far too great to permit almost any kind of an observation whatever, and, in addition to this difficulty, the protozoa immediately become decidedly abnormal and die very quickly. The best Swezy could do was keep the protozoa alive for six hours, so she states. This investigator also used physiological salt solution to dilute the intestinal contents and states that "the flagellate tends to round up immediately, becoming greatly distended and dissolving a few minutes after being placed on the slide." The osmotic pressure of such a solution is much too high for *Trichonympha* but not for *Trichomonas* from the same host. Half this amount of salt is far better.[1] But better still is:

$NaCl$	0.3	grams
$CaCl_2$	0.02	"
KCl	0.02	"
$MgCl_2$	0.01	"
NaH_2PO_4	0.001	"
$NaHCO_3$	0.01	"
H_2O	100 cc.	
Löffler's blood serum	0.5	grams.

This is filtered and used immediately. Instead of the serum, 5–10 grams of fresh *Termopsis* feces may be thoroughly shaken up in the above mixture, and may be used with or without filtering. If filtered, a small amount (1–2 grams) of finely powdered lignocellulose is added. In this way, many *Trichonympha* live ten days and longer and may be observed as constantly as desired, under a sealed cover glass or otherwise.

Method of Food Ingestion.

When the flagellate takes in food, the posterior end, owing to contraction of the longitudinal endoplasmic myonemes, becomes invaginated, thus forming a cavity or temporary cytostome (Fig. 6). These myonemes, about two micra in breadth and easily observed in living material, are the principal organelles concerned with body movement. They begin very near the anterior tip (somewhere near, possibly within the centroblepharoplast) and

[1] More recent observations show that 0.2 per cent. NaCl is the best salt solution for observing the protozoa of certain tropical termites.

run almost parallel, widening out slightly posteriorly, for approximately three fourths the body length (Fig. *A*). Posterior to their termination, there is a hyaline, much less differentiated region, void of fibers, flagella and myonemes. This region flows backwards and forwards (Fig. 8) with much freedom of movement

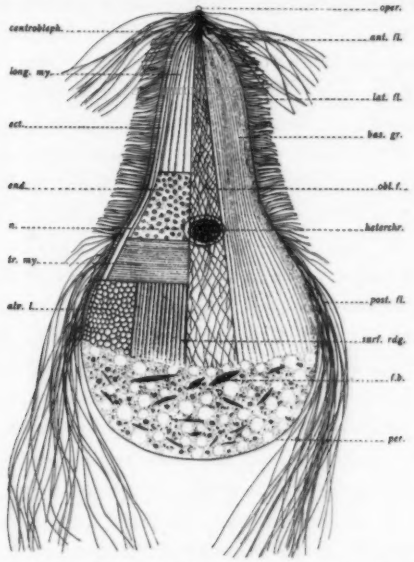

FIG. *A*. Diagrammatic figure of *Trichonympha campanula*. Sections of the body show the structures found at different levels. Surface ridges form the outer layer with their rows of flagella; beneath are successively the oblique fibers, alveolar layer and transverse myonemes. In the endoplasm are the longitudinal myonemes.

Abbreviations: *alv. l.*, alveolar layer; *ant. fl.*, anterior zone of flagella; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f.b.*, food bodies; *heterch.*, heterochromosome; *lat. fl.*, lateral zone of flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operculum; *per.*, periplast; *post. fl.*, posterior zone of flagella; *surf. rdg.*, surface ridges; *tr. my.*, transverse myonemes. × 300. (Figure and legend after Kofoid and Swezy.)

when compared to the rest of the body. It, in fact, sometimes extends posteriorly more than twice the distance shown in Fig. 8. At times great masses of wood particles are seen adhering to the outer (and probably sticky) surface of the body in this region. They enter the body when the posterior end invaginates.

The Successive Stages in Food Ingestion.—A flagellate just previous to taking in food (Fig. 1) usually has few wood particles inside the body; whereas at other times about one third (the posterior third) of the body is always filled with the wood particles. First the longitudinal myonemes contract, thus causing the posterior end to become more rounded and slightly flattened centrally (Fig. 2). It is quite possible that the transverse myonemes expand at the time, though it has been impossible to actually observe this. In the next stage (Fig. 3) the body is more contracted and hence shorter; this contraction, owing to the fact that the inner or central portion was pulled forward while the outer and more rigid ectoplasmic portion did not invaginate, results in the formation of a cup-shaped cavity (Fig. 5) lined with the wood particles which adhered to the outer and undifferentiated region prior to invagination. Now the cytoplasm of the most posterior portion of the undifferentiated region soon begins to flow backward, thus narrowing the posterior end of the cavity (Fig. 6). This continues until finally the cavity is completely closed, the wood particles remaining inside (Fig. 7).

Other *Trichonympha* sometime get caught in the cavity just as it begins to close (Fig. 9), and may be seen swimming in this position for ten minutes or more, for caught individuals free themselves with great difficulty. This, of course, is not a common occurrence, though five such observations were made within four hours. In three termites, out of more than 500 that were examined, perhaps half the *Trichonympha* present had other *Trichonympha* partially ingested and securely fastened inside their bodies. The cause of this peculiar phenomenon is entirely unknown. Living individuals of *Trichomonas termopsidis*, a much smaller flagellate always associated with *Trichonympha*, are sometimes ingested along with wood particles, though not in sufficient quantity perhaps to be of much food value. *Leidyopsis sphærica*, another protozoön closely related to and associated with *Trichonympha* in *Termopsis angusticoliis*, takes in wood particles similarly to the method described here. It has also been observed killed and half ingested by *Trichonympha*. This, however, is a rare observation.

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Explanation of Plate 1.

Figs. 1–7. Method of wood ingestion in *Trichonympha campanula*. All figures × 180.

Fig. 1. Before the ingestion of food (wood). Note the particles of wood adhering to the posterior surface of the body.

Fig. 2. First stage in the process of ingestion. The longitudinal myonemes (see text Fig. *A*) have contracted and the posterior end has thus become flattened with the wood particles sticking to the outer and probably sticky surface of the body.

Fig. 3. By greater contraction of the longitudinal myonemes a cup-shaped cavity is being formed by an invagination of the posterior end.

Fig. 4. Cup-shaped cavity or temporary cytostome completely formed.

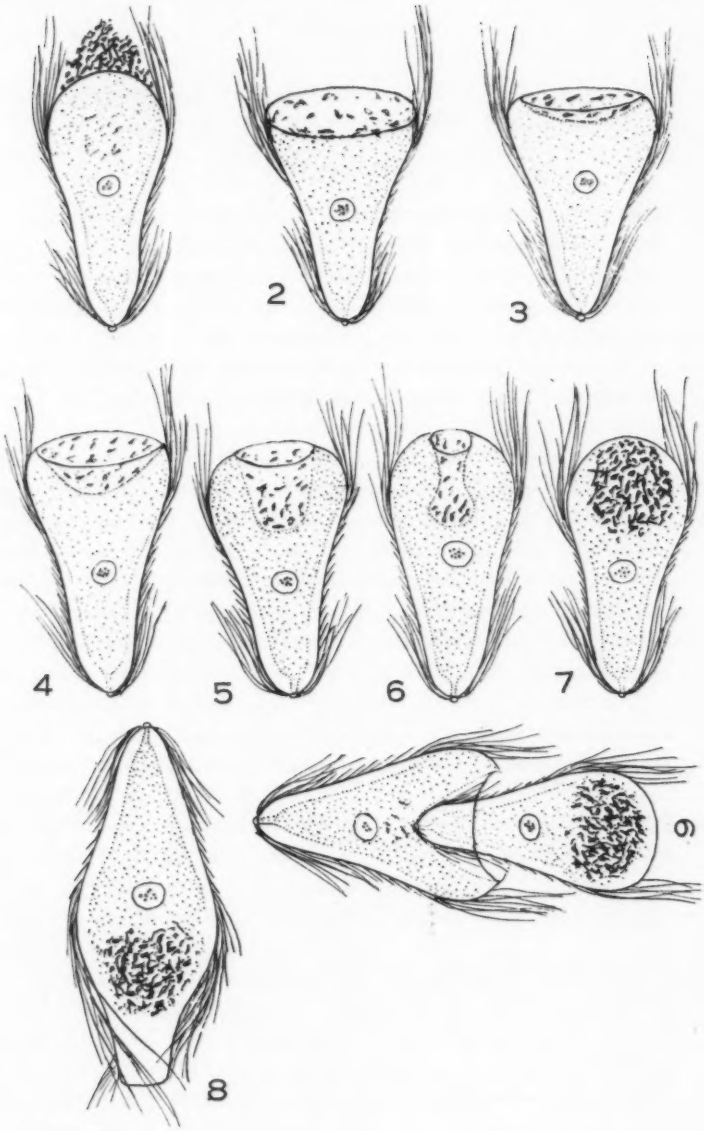
Fig. 5. The cavity is closing by a flowing together or backward of the clear, hyaline and undifferentiated portion of the body. Protoplasm in this region is free to flow back and forth for a considerable distance. When very large pieces of wood are ingested, it flows back around them pseudopodia-like; but this is unusual.

Fig. 6. A later stage in the closing of the cavity.

Fig. 7. The cavity has closed and the wood particles have been ingested.

Fig. 8. Shows the cytoplasm of the posterior end flowed backwards for a considerable distance.

Fig. 9. One *Trichonympha* caught in the temporary cytostome of another and struggling to free itself.



L. R. CLEVELAND.

THE ABILITY OF TERMITES TO LIVE PERHAPS INDEFINITELY ON A DIET OF PURE CELLULOSE.

L. R. CLEVELAND.[1]

It seems quite certain from the work that has been done that the number of animals capable of using cellulose, along with other material, to any considerable extent is quite small indeed. This question, however, has been crucially investigated in few, if any, instances. For example, we are told that the goat can utilize a fairly high percentage of the cellulose content of its diet; the cow and the horse a smaller percentage; and man so small a quantity that it need not be considered. In most of the experiments which have been done on these animals, no distinction whatever was made between cellulose and hemicellulose, and the latter, which is perhaps more easily digested, was certainly present in a far greater quantity. In brief, we do not know whether cellulose can be used at all or not. The general belief is that it cannot, except perhaps in the case of some xylophagous insects. But the ability of these insects to utilize cellulose has been little investigated. Certainly, no animal—insect or what not—has been shown to be able to live on it. In the present paper the ability of an insect to maintain itself on a cellulose diet for twelve months and longer is demonstrated beyond question.

Most termites in nature feed solely on wood, which is always as much as 50 per cent. cellulose. This, then, coupled with the fact that they may be easily collected in great abundance and kept in the laboratory indefinitely, makes them ideal for the study of cellulose digestion.

Several thousand termites from two genera, *Termopsis* Heer and *Reticulitermes* Holmgren, were collected. *Termopsis* is the large Pacific Coast termite and is found in abundance in dead logs and stumps in California, Washington, Oregon, New Mexico and Arizona. *Reticulitermes* is a smaller form, with many species,

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some of which are present in almost any locality in the United States and many other countries. Material for this study was collected in Maryland. *Termopsis* belongs to the family Kalotermitidae and *Reticulitermes* to the family Rhinotermitidae. So, the two genera selected for experimentation are quite different in structure, habits, location, etc.

Cellulose from two sources, cotton and wood, was used. That from cotton was the well-known Whatman filter paper No. 43, which is extracted in hydrochloric and hydrofluoric acids and in ether and contains .00006 per cent. ash. But cellulose prepared in this way is subjected to drastic treatment, which might render it more open to attack. In order to test this, fresh western yellow pine wood was made into sawdust, which was dried and ground so as to pass through a 50-mesh screen. This was extracted with alcohol and benzene and boiled under a reflux-condenser—Dore in a personal communication ('20) suggested the use and preparation of lignocellulose to me—for six hours, some in 5 per cent. HCl and some in 5 per cent. NaOH. In the material prepared in this manner, lignin and cellulose, with perhaps a trace of mannose, remain. But lignin has no food value, for when it alone is fed termites they do not live any longer than when they are starved. Hence we are dealing only with the cellulose content of this material, and results obtained by feeding it may be compared in every way with those obtained from feeding the cotton cellulose.

Experimental.

a. The Genus Termopsis.

The experiments were each carried out in two parts, which were duplicates except for food. In part one the food was lignocellulose and in part two it was cotton cellulose in the form of Whatman filter paper No. 43.

In each part of the first experiment, approximately 500 individuals, some soldiers but mostly nymphs, were placed in a large glass vessel which just fitted in a moist chamber. From time to time more food as needed was placed in the glass vessel and occasionally the fecal debris was removed. In the moist chamber around the outer edge of the glass vessel which con-

tained the termites and their food, a small amount of water was added every ten days or so. In this way the food was constantly kept sufficiently moist to make it wholesome and yet not so moist that the termites would be injured by the growth of molds on their fecal pellets.

In each part of the second experiment, 10 nymphs were placed in each of ten large size sputum jars, which were kept in a moist chamber. Moisture and food were added as in the first experiment. This experiment differs from the first chiefly in the size of the vessel which contained the termites and in the number of termites which were placed in each vessel.

In each part of the third experiment, approximately 100 nymphs with food were placed in a liter Erlenmeyer flask. This flask was connected by a capillary tube to a similar flask which contained a little water. The air and water were changed every 60 or 70 days, but the flask which contained the termites was never opened, for sufficient food was added when the experiment was started to last to date—one year—and longer.

b. The Genus Reticulitermes.

This genus contains many individuals—half the colony at least—of the true worker caste, while *Termopsis* does not have any true workers. In each experiment, workers, soldiers, and nymphs were used.

Three experiments were carried out, each in two parts, using lignocellulose in part one and cotton cellulose in the form of Whatman filter paper No. 43 in part two, just as with *Termopsis*, except that in the third experiment four flasks, with approximately 500 termites in each, were used.

Results.

So far as it is possible to determine, these termites on a pure cellulose diet have behaved in every way exactly as the controls, that have been given a wood diet and kept in the same manner. No difference whatever has been noticed between those that received a diet of cotton cellulose (Whatman filter paper No. 43) and those that received a diet of wood cellulose (lignocellulose). The drastic treatment to which the cotton cellulose is subjected during preparation seemingly, then, does not render it more

open to attack. At any rate, the lignocellulose, subjected to much less drastic treatment, is just as open to attack.

Everything that has been observed in termites in nature during a year's time, has been observed in these cellulose-fed termites. Many of the nymphs have become sexually mature young adults and have laid a very large number of eggs, which have hatched normally. Larvæ from these eggs have grown even more rapidly than those from the eggs of some of the wood-fed controls. In all the experiments, the larvæ now present are greater both in number and in actual body weight than those individuals which were present in the beginning. In other words, the total weight of the colony is now more than twice what it was when the experiment was begun. This increase which has taken place on a cellulose diet is as great as that which has taken place on a wood diet. No deaths have occurred. Molting and the formation of winged adults have been observed in each experiment. Cellulose, then, in every noticeable way, has been as nutritious, so far (12 months), as the normal diet of wood.

Discussion.

If these insects can maintain themselves in a perfectly normal manner indefinitely on a cellulose diet, they must be able in some way to fix atmospheric nitrogen, which they use in manufacturing proteins; or else, contrary to the current opinion, they must be able to transform carbohydrates into proteins. These two possibilities are now being investigated from many angles.

It should be mentioned, for the benefit of those who have not seen the earlier papers of the writer ('23*a*, '23*b*, '24*a*, '25*a*, '25*b*) on the symbiosis between termites and their intestinal protozoa, that termites from which the protozoa have been removed by either of the three methods (incubation, starvation, oxygenation) lose the ability to live on cellulose or on wood. However, when the protozoa are restored, *i.e.*, when the defaunated termites are reinfected, the ability to live on cellulose or on wood is regained. The teeming menagerie of intestinal protozoa which the termites harbor, then, either digest the cellulose *in toto* or else play a very important part in its digestion.

ADDENDUM.

It is now eighteen months since these experiments were started. The termites appear perfectly normal in every way. Thousands of eggs have been laid during the past week, and several winged forms have been produced. In some cellulose-fed artificial colonies which contained ten adult individuals in the beginning more than two hundred half-grown individuals are now present. Thus, the weight of these colonies has increased more than forty times on a diet of cellulose.

Several attempts have been made to determine whether or not atmospheric nitrogen is being fixed. The respiratory quotient has been measured and, as would naturally be expected on a carbohydrate diet, has been found to be practically 1/1. When termites (*Termopsis*) are confined in air with barometric changes being noted and temperature being kept constant, a negative pressure is very soon developed. This indicates that nitrogen is being fixed, but analyses of air samples taken from tubes where the negative pressures have developed have shown very little, if any, change in the nitrogen percentage.

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